Communication

Modes of Fatty Acid Desaturation in Cyanobacteria: An Update

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Abstract: Fatty acid composition of individual species of cyanobacteria is conserved and it may be used as a phylogenetic marker. The previously proposed classification system was based solely on biochemical data. Today, new genomic data are available, which support a need to update a previously postulated FA-based classification of cyanobacteria. These changes are necessary in order to adjust and synchronize biochemical, physiological and genomic data, which may help to establish an adequate comprehensive taxonomic system for cyanobacteria in the future. Here, we propose an update to the classification system of cyanobacteria based on their fatty acid composition.

Keywords: cyanobacteria; fatty acids; fatty acid desaturases; desaturation; lipids; taxonomy

1. Introduction

Cyanobacteria (formerly—blue-green algae) are considered as one of the most ancient groups of living organisms on Earth [1]. Studies of fossil microorganisms in Precambrian rocks (3.5–0.5 billion years ago) indicated the temporal morphological changes in fossil cyanobacterial communities caused by the irreversible changes of physicochemical conditions on Earth [2,3]. Different species of modern cyanobacteria inhabit almost all environments—from soil to fresh and sea waters, as well as such extreme habitats as hot springs, soda and salt lakes, *etc.* The morphology of some species, especially, extremophilic ones, resemble that found in fossils. Such species are called the relict cyanobacteria [2,4]. A comparison of artificial systems consisting of modern prokaryotes, including extremophilic

cyanobacteria, and Proterozoic forms of cyanobacterial communities suggested that the cyanobacteria are very conservative and have changed insignificantly morphologically and, probably, physiologically during the past, at least, 2 billion years [4]. These negligible changes also refer to the membrane system of cyanobacteria, which is mainly determined by the lipids and fatty acid (FA) species.

The membranes of cyanobacteria are represented by the cytoplasmic (plasma) membrane and thylakoid membranes. Both membranes contain four major glycerolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). The molecular motion of these glycerolipids is determined mainly by the extents of unsaturation of the fatty acids that are esterified to the glycerol backbones [5]. The extent of unsaturation is, in turn, determined by the activity of fatty acid desaturases, the enzymes that introduce double bonds into specific positions of fatty-acyl chains of lipids [6]. Changes in the unsaturation of FAs affect various functions of membrane-bound proteins, such as the photochemical and electron-transport reactions that occur in thylakoid and cytoplasmic membranes of cyanobacterial cells [7].

FA composition of lipids of cyanobacteria is determined by the chain length (number of carbon atoms) and number of double bonds in these chains. In cyanobacteria, the FA chain length usually varies from C_{14} to C_{18}. The number of double bonds in these chains may vary from 0 to 4 providing fully saturated FAs (with no double bonds), monoenoic (with 1 double bond), dienoic (with 2 double bonds), trienoic and tetraenoic (with 3 and 4 double bonds, respectively) FAs. FA composition of individual species of cyanobacteria is so conserved, that it may be used as a phylogenetic marker [8–10].

The system of classification of cyanobacteria according to their FA composition was proposed by Kenyon [11,12] and improved by Murata and co-workers [13]. According to this Kenyon-Murata classification, all cyanobacterial strains are divided into four distinct groups. Organisms in Group 1 introduce only one double bond at the Δ^9 position of fatty acids (usually C_{16} or C_{18} FAs) esterified at the *sn-l* position of the glycerol moiety. In cyanobacteria of Group 2, the C_{18} stearic acid is desaturated at the Δ^9, Δ^{12}, and Δ^{15} [13] positions and the C_{16} palmitic acid is desaturated at the Δ^9 and Δ^{12} positions. In Group 3, the C_{18} acid is desaturated at the Δ^6, Δ^9, and Δ^{12} positions. Finally, in Group 4, the C_{18} stearic acid is desaturated at the Δ^6, Δ^9, Δ^{12}, and Δ^{15} positions (Table 1) [13].

The available experimental data on desaturation in cyanobacterial cells suggest that the $\Delta9$-desaturase counts the carbon number from the carboxyl terminus, whereas the so-called $\Delta15$-desaturase is, in fact, the $\omega3$-desaturase, which counts the carbon number from the methyl-terminus [14]. Although significant progress has been made in understanding the molecular basis of regiospecific desaturation by soluble acyl–acyl-carrier-protein desaturases [15] the counting order of the acyl-lipid membrane-bound $\Delta12$-desaturase is still under question. It is also important to note that the $\Delta15(\omega3)$-desaturase of the cyanobacterium *Synechocystis* sp. PCC 6803 cannot introduce double bonds into Δ^9 monoenoic FAs, and it requires $\Delta^{9,12}$ dienoic substrate for its activity [16]. Here, we will use the Δ^x abbreviation system to simplify the designations.

Current conclusions on modes of FA desaturation in cyanobacteria are solely based on biochemical analysis of FAs and lipid classes [8–14]. Modern advances in sequencing techniques allowed determination of the whole genomes of various cyanobacterial strains. The genes for the specific acyl-lipid fatty acid desaturases have been identified in many cyanobacterial species [17]. This supports a need to update the previously postulated FA-based classification of cyanobacteria. Here, we propose an updated grouping

of cyanobacteria, according to their FA composition, based on recent findings in cyanobacterial genomics and biochemistry.

Table 1. Fatty-acid composition of the total lipids from various cyanobacterial strains (adapted from Murata *et al.* 1992 [13]).

Organism		Fatty Acids										
		14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	α18:3	γ18:3	18:4
			Δ^9		Δ^9	$\Delta^{9,12}$		Δ^9	$\Delta^{9,12}$	$\Delta^{9,12,15}$	$\Delta^{6,9,12}$	$\Delta^{6,9,12,15}$
Group 1												
Mastigocladus laminosus	F	+	−	+	+	−	+	+	−	−	−	−
Synechococcus PCC 7942	U	+	−	+	+	−	+	+	−	−	−	−
Synechococcus PCC 6301	U	+	−	+	+	−	+	+	−	−	−	−
Synechococcus lividus	U	−	−	+	+	−	+	+	−	−	−	−
Group 2												
Plectonema boryanum	F	+	−	+	+	−	+	+	+	+	−	−
Nostoc muscorum	F	+	−	+	+	−	+	+	+	+	−	−
Anabaena variabilis	F	−	−	+	+	+	+	+	+	+	−	−
Synechococcus PCC 7002	U	+	−	+	+	−	+	+	+	+	−	−
Group 3												
Arthrospira platensis	F	+	+	+	+	−	+	+	+	−	+	−
Synechocystis PCC 6714	U	+	+	+	+	−	+	+	+	−	+	−
Group 4												
Tolypothrix tenius	F	−	−	+	+	−	+	+	+	+	+	+
Synechocystis PCC 6803	U	−	−	+	+	−	+	+	+	+	+	+

PCC—Number in P̲asteur C̲ulture C̲ollection. F—filamentous species; U—unicellulae species.

2. Results and Discussion

2.1. Cyanobacteria of Group 1

The organisms of Group 1 synthesize only monoenoic FAs usually desaturated at Δ^9 position. This group is presented by mesophilic and thermophilic strains of unicellular freshwater *Synechococcus* and *Cyanobacterium*, as well as by ramified filamentous heterocystous thermophilic *Mastigocladus laminosus* [18,19]. Previously, it was suggested that the number of double bonds in FA chains correlates with complexity of cyanobacterial cells [10–12], and filamentous strains are not distributed in Group 1. However, it appeared that *Mastigocladus laminosus* also belongs to Group 1 [13,20]. Thus, organisms that synthesize monoenoic fatty acids (usually, $14:1\Delta^9$, $16:1\Delta^9$, and $18:1\Delta^9$) may be represented by unicellular and filamentous species (Table 2).

Genomic sequencing and biochemical analysis revealed that desaturation at Δ^9 position may be performed by different isozymes of Δ9-desaturase. Some of these isozymes may be specific to *sn*-1 or *sn*-2 positions of the glycerol moiety [21].

Table 2. An updated classification of cyanobacteria on the basis of their fatty acid composition.

Organism	14:0	14:1 Δ^9	16:0	16:1 Δ^9	16:2 [e] $\Delta^{9,12}$	18:0	18:1 Δ^9	18:2 $\Delta^{9,12}$	α18:3 $\Delta^{9,12,15}$	γ18:3 $\Delta^{6,9,12}$	18:4 $\Delta^{6,9,12,15}$
Group 1											
Synechococcus elongatus PCC 7942 [a]	−	−	+	+	−	+	+	−	−	−	−
Mastigocladus laminosus	−	−	+	+	−	+	+	−	−	−	−
Synechococcus lividus	−	−	+	+	−	+	+	−	−	−	−
Synechococcus vulcanus	+	+	+	+	−	+	+	−	−	−	−
Cyanobacterium stanieri PCC 7202 [a]	−	−	+	+	−	+	+	−	−	−	−
Cyanobacterium sp. B−1200 [b]	+	+	+	+	−	+	+	−	−	−	−
Synechococcus cedrorum	−	−	+	+	−	+	+	−	−	−	−
Group 2											
Prochlorococcus marinus [c]	−	−	+	+	+	+	+	+	−	−	−
Synechococcus sp. (marine) [d]	−	−	+	+	−	+	+	+	−	−	−
Prochlorothrix hollandica [e]	+	+	+	+	+	+	+	+	−	−	−
Group 3α											
Leptolyngbya boryana	−	−	+	+	−	+	+	+	+	−	−
Nostoc sp.	−	−	+	+	−	+	+	+	+	−	−
Anabaena sp. [f]	−	−	+	+	−	+	+	+	+	−	−
Synechococcus sp. PCC 7002 [a]	−	−	+	+	−	+	+	+	+	−	−
Gloeobacter violaceus	−	−	+	+	−	+	+	+	+	−	−
Trichodesmium erythraeum	−	−	+	+	−	+	+	+	+	−	−
Group 3γ											
Arthrospira platensis B-256 [b]	+	−	+	+	−	+	+	+	−	+	−
Synechocystis sp. PCC 6714 [a]	−	−	+	+	−	+	+	+	−	+	−
Synechocystis sp. B-274 [b]	−	−	+	+	+	+	+	+	−	+	−
Group 4											
Tolypothrix tenius	+	+	+	+	+	+	+	+	+	+	+
Synechocystis sp. PCC 6803 [a]	−	−	+	+	−	+	+	+	+	+	+
Lyngbya sp. PCC 8106 [a]	−	−	+	+	−	+	+	+	+	+	+
Nodularia spumigena	−	−	+	+	−	+	+	+	+	+	+

[a] Number in Pasteur Culture Collection (PCC); [b] Number in the Collection of Microalgae and Cyanobacteria of the Institute of Plant Physiology RAS (IPPAS); [c] *Prochlorococcus* strains NATL1A, MIT 9211, MIT 9301, MIT 9303, MIT 9312, MIT 9313, MIT 9515, AS9601, CCMP1375, CCMP1986, *etc*; [d] Marine species of *Synechococcus*: strains BL107, CC9311, CC9605, CC9902, RCC307, RS9917, WH5701, WH7805, WH8102, *etc.*; [e] *Prochlorothrix hollandica* was reported to have Δ9- and Δ4-desaturase activities [22]; [f] At least, 9 species of *Anabaena* were studied [23].

The presence of six genes for the Δ9-desaturases in the genome of *Gloeobacter violaceus* [24] suggests that some isozymes may be specific both to the *sn*-position and to the carbon chain length of FAs. Since *Nostoc* [21] and *Gloeobacter* [24] do not belong to Group 1, one may suggest that multiple isoforms of the Δ9-desaturase are not typical to cyanobacteria of Group 1. Indeed, the type strains of unicellular freshwater *Synechococcus*, *Synechococcus elongatus* PCC 7942 (NCBI Reference Sequence

NC_007604) and *Synechococcus elongatus* PCC 6301 (NC_006576), each have only one gene for the $\Delta 9$-desaturase. The appearance of $18:1\Delta^9$ and $16:1\Delta^9$ at *sn*-1 and *sn*-2 in these two strains [13] suggests that their $\Delta 9$-desaturases are not specific to the chain length of FAs and to the *sn*-position. However, the genome of a thermophilic unicellular cyanobacterium, *Thermosynechococcus elongatus* (similarly to filamentous *Nostoc* [21], and unusual unicellular "*single-membrane*" organism, *Gloeobacter* [24]) also carries several copies (three) of a gene for the $\Delta 9$-desaturase.

The alignment of amino acid sequences of $\Delta 9$-desaturases from various strains of cyanobacteria revealed that these enzymes can be classified into three groups (Figure 1). The first group, DesC1, is represented by the enzymes that are similar to the $\Delta 9$-desaturase, which is specific to *sn*-1 position of glycerolipids in *Synechocystis* sp. PCC 6803 and *Anabaena variabilis* [25]. Second group, DesC2, forms a cluster of enzymes homologous to the $\Delta 9$-desaturase, which is specific to *sn*-2 position in Antarctic *Nostoc* sp. 36 [21]. Differences in specificity of DesC1 and DesC2 to *sn*-position were demonstrated in accurate biochemical experiments [21,25]. The third distinct group of $\Delta 9$-desaturases, DesC3, is clustered by four amino acid sequences that were deduced from the genomic data of *Gloeobacter violaceus* [24] and two sequences of other cyanobacterial species.

At least four conservative His-containing domains found in these three groups of $\Delta 9$-desaturases. DesC1 and DesC2 were more similar to each other in amino acid sequences than DesC3 (Figure 1). First, second, and fourth His-containing domains (HRLXXHRSF, GHRXHH, GESWHNNHHA) are rather conservative in all three groups of $\Delta 9$-desaturases. The major differences in amino and sequences were observed in the domain 3 of DesC3 if compared to DesC1 and DesC2. The latter two have a very conservative third domain HFTWFVNSATH, while DesC3 has no His residues in this region. Conservative histidine residues function as coordinators of a diiron cluster in the active center of a desaturase that performs dehydrogenation reactions resulting in the formation of double bonds in the FA chains. Therefore, the positioning of His residues affects the specificity of FA desaturases in terms of a chain length and a position of desaturation [26]. The structural basis for positional specificity of desaturases is unknown. It might appear that the ability of desaturases to recognize a certain *sn*-position is similar to that of glycerolipid acyltransferases, in which a $H(X)_4D$ motif is a critical component for the enzyme's activity [27].

The specificity of DesC1 and DesC2 to *sn*-1 and *sn*-2 positions have been documented [21,25], the specificity of DesC3 group of $\Delta 9$-desaturases was not studied experimentally. Therefore, the exact function of this type of enzymes is unknown. Chi *et al.* [17] found that this group of desaturases resembles a large family of membrane-associated $\Delta 5$- or $\Delta 9$-desaturases. Analysis of FA composition of *Gloeobacter violaceus* did not reveal any $\Delta 5$-desaturated FAs [28,29]. So, this should be some $\Delta 9$-desaturase with yet unraveled activity and specificity.

Accession number	Species	Amino acid sequence	AA count

Domain H1   Domain H2

DesC1

WP_010873728	*Synechocystis* sp. PCC 6803	FSWKAVGMAFLLYVITGGIGITLGFHRCISHRSFNVPKWLEYIFVICGTLACQGGVFEWVGLHRMHHKFSDTTPDPHDSNK-GFWWSHIGWM	129
WP_010995768	*Anabaena* sp. PCC 7120	FSWAAVGVALLLYWITGGLGITLGFHRLVTHRSFQTPKWLEYFLVLCGTLACQGGPIEWVGTHRIHHLHSDTDPDPHDSNK-GFWWSHIGWL	125
YP_007164144	*Cyanobacterium stanieri* PCC 7202	FSWGAVGLFFVLYWLTACIGITLGFHRLVSHRSFETPKWLEYLLVLCGSLACQGGPIQWVGLHRVHHKFSDHDGDPHDSNK-GFWWSHMGWM	125
WP_002733282	*Microcystis aeruginosa* PCC 7005	FSWGAVGVTLILYWITGGLGITLGFHRLVSHRSFKTPKWLEYFLVLCGTLACQGGAIAWIGLHRLHHKYSDTAPDPHDSNK-GFWWSHMGWM	125
WP_006199118	*Nodularia_spumigena* CCY9419	FSWNAVGVALLLYWITGGLGITLGFHRLVTHRSFQTPKWLEYFLVFCGTLACQGPIEWIGTHRIHHVHSDTDPDPHDSNK-GFWWSHIGWL	125
WP_006616281	*Arthrospira platensis* NIES-39	FSWAAIGVAVFLHWVTGGLGITLGFHRLITHRSFETPKWLEYFLAFCGALACQGGPIQWVGLHRMHHLYSDQTLDPHDSNK-GFWWSHMGWM	125
WP_006633584	*Microcoleus vaginatus* FGP-2	FSWSAVGLAVFLHWVTGGLGITLGFHRLVTHRSFQTPKWLEYFLVICGTLSCEGGPIDWVGMHRLHHIHSDTETDPHDSNQ-GFWWSHMGWM	125
WP_011141235	*Gloeobacter violaceus* PCC 7421	FSWSALALAIFLHWALGCLGITLGFHRLLSHRSFTVPKPLEYFLALLGTLCLQGGPVLWVAHHRLHHAHSDTEADPHDSNR-GFWWSHWGWM	125
WP_011320892	*Anabaena variabilis* ATCC 29413	FSWAAVGVALLLYWITGGLGITLGFHRLVTHRSFQTPKWLEYFLVLCGTLACQGGPIEWVGTHRIHHLHSDTDPDPHDSNK-GFWWSHIGWL	125
WP_011611109	*Trichodesmium erythraeum* IMS101	FSWTAVGLALLLHWVTGGLGITLGFHRMLTHRSFKTPKWLEYFFIFCGTLACEGGVCDWVGLHRLHHQHSDNEQDPHDSHR-GFWWSHMGWM	125
WP_012412456	*Nostoc punctiforme* PCC 73102	FSWTAVGVGFLLYWVTGGLGVTLGFHRLVTHRSFQTPKWLEYLLVFFGTLSCQGGPIEWIGTHRIHHLNSDTDTDPHDSNK-GFWWSHMGWL	125
WP_015170515	*Geitlerinema* sp. PCC 7407	FSWAAVGVALFLHWVTGGLGITLGLHRLVAHRSFQTPKWLEYFLVFCASLAAQGGPIEWVGLHRIHHLHSDQSVDHHNSGK-GFWWSHMGWL	125
WP_015177398	*Oscillatoria nigro-viridis* PCC 7112	FSWSAVGLAVFLHWVTGGLGITLGFHRLVTHRSFQTPKWLEYFLVICGTLSCEGGPIDWVGMHRLHHIHSDTETDPHDSNQ-GFWWSHMGWM	125
WP_015217907	*Cyanobacterium aponinum* PCC 10605	FSWGAVGITAFLYWLTACIGVTLGYHRLVSHRSFETPKWLEYFLVFCGTLACEGGPISWVGLHRIHHKYSDHEGDPHDSNK-GFWWSHMGWM	126
WP_021836317	*Crocosphaera watsonii* WH 0401	FSWQAVGVTLLLYWVTGALGITLGFHRLVSHRSFNSPKWLEYLLVFFGTLACQGGPIQWVGLHRIHHKYSDTEPDPHDSNK-GFWWSHMAWM	125
WP_027401795	*Aphanizomenon flos-aquae* NIES-81	FSWQAVGVALLLYWITGGLGITLGYHRLVTHRSFQTPKWLEYVLVIFGTLSCEGGPIEWVGTHRIHHLHSDTESDPHDSNQ-GFWWSHMGWM	124
WP_026099734	*Prochlorothrix hollandica* PCC 9006	FSWYAVALALFMHWFTGCLGITLGWHRLISHRSFQVPKWLEYFFVFCGSLSCQSGPIEWIGLHRHHHAYSDQEEDHHNSNK-GFWWSHMNWM	125
AAB61353	*Synechococcus* sp. PCC 7002	FSWQAVGVFLLFHWITGGIGITLGFHRLVSHRSFEVPKWLEYFLIFCGTLACQGGPIDWIGLHRIHHKHSDNTPDPHDSNK-GFWWSHIGWM	125
YP_401578	*Synechococcus elongatus* PCC 7942	FNWPAVGVMVALYYITGCFGITLGWHRLISHRSFEVPKWLEYVLVFCGTLAMQHGPIEWIGLHRHHHLHSDQDVDHHDSNK-GFLWSHFLWM	125

DesC2

CAF18426	*Nostoc* sp. 36	FSWSALGLLVFLHWLFGSIGICLGYHRLLSHKSFQVPKWLEYAIALIGALALQGGPIFWVGGHRQHHAHTEDIDLDPYSAQKGFWWSHILWI	131
AAD00699	*Thermosynechococcus vulcanus*	FSWSALAVTIFLHWLFGSIGICLGYHRLLSHRSFQVPQWLEYVIAVVGALAMQGGPIFWVAGHRLHHAHTEDEIKDPYSARRGFWWSHMLWL	127
WP_026734531	*Fischerella* sp. PCC 9605	FSWSALGITIFLHWLFGSIGICLGYHRLLTHRSLHVPKWLEYAIATLGALAIQGGPIFWVATHRLHHAHTEDEDKDPYSSRRGFWWSHMLWI	129
WP_011057556	*Thermosynechococcus elongatus* BP-1	FSWSALAVTIFLHWLFGSIGICLGYHRLLSHRSFQVPQWLEYVIAVVGALAMQGGPIFWVAGHRLHHAHTEDEIKDPYSARRGFWWSHMLWL	127
WP_012307598	*Synechococcus* sp. PCC 7002	FSWSAIAVCLFLHWLFGSIGICLGYHRLLSHKSFKVPQWLEYTLAFIGALALQGGPIFWVAGHRLHHAHTEDEVKDPYSAKRGFWWSHMLWI	128
WP_015171756	*Geitlerinema* sp. PCC 7407	FSWSALGVMLFLHWLFGSIGICLGYHRLLSHRSLRVPKPLEYTFGLLGALALQGGPIFWVAGHRLHHAFTEDVHKDPYSARRGFWWSHILWI	131
WP_015172022	*Geitlerinema* sp. PCC 7407	FSWSALGVMLGLHWLFGSIGICLGYHRLLSHRSFRVPRWLERSIALLGALALQGGPIFWVAGHRLHHAYTEDEQKDPYSARRGFWWSHVLWI	128
WP_015175264	*Oscillatoria nigro-viridis* PCC 7112	FSWSALGMTLFLHWLFGSIGICLGYHRLLSHRSFQVPKGLEYAIALIGATALQGGPIFWVAGHRLHHAYTEDVDKDPYSARRGFWWSHMLWI	128
WP_015186765	*Gloeocapsa* sp. PCC 7428	FSWSALGVTLFLHWLFGSIGICLGYHRLLSHRSLQVPKWVEYAIATCGALAIQGGPIFWVAGHRLHHAHTEDNDKDPYSSRRGFWWSHMLWI	130
WP_017286722	*Leptolyngbya boryana* PCC 6306	FSWKALGVMLLLHWLFGSIGICLGYHRLLTHRSLQVPKPLEYVLTIIGTLAMQGGPIFWVAGHRMHHLHTEDVDNDPYSAQKGFWWSHMLWV	129
WP_019499810	*Pseudanabaena* sp. PCC 6802	FSWSALIATIFLHWLFGSIGICLGYHRLLSHRSFQVPKWLEYAIALIGALALQGGPIFWVAGHRLHHAHTEDIEQDPYSARRGFWWSHMLWI	128
WP_026148909	*Leptolyngbya boryana* PCC 6306	FSWSALGTMLVLHWFFGSIGICLGYHRLLSHRSFQVPKGLEYAIALIGALALQGGPIFWVAGHRLHHAHTEDEEKDPYSARRGFWWSHMLWI	127

DesC3

WP_011142860	*Gloeobacter violaceus* PCC 7421	PPGPIEGSLLLGLWLVTGFGVTVGYHRLFTHRSFSAAPWVQALLAVSGSMAGQGSVISWVALHRRHHELSDQPGDPHSPNLHGQDPVGRLRG	149
WP_011141944	*Gloeobacter violaceus* PCC 7421	PPGAVEVGLFLGGFLCTGFGVNIGLHRHFTHRSFQAVTPVRVALAILGSMAAQGPVIFWVALHRLHHEFSDQPGDIHSPHFHGEGWWGSVRG	145
WP_011141938	*Gloeobacter violaceus* PCC 7421	PLRLLDVALLLGMYVLTVFGLEVGYHRHFAHGAFKASPPVRVTLAVLGGMAASGPLIYWAATHRLHHHFPDEPGDPHSPYLKADRPLERLPG	157
AAS98775	*Lyngbya majuscula*	PISSVEIGLLIGMWALSMIGMSVGLHRYFAHRAFKTSQTMSVILAILGCMGAQGPVVSWVAVHRRHHEYSDLPGDPHSPNPELLGEGIFGTG	149
WP_008184032	*Moorea producens* 3L	PISSVEIGLLIGMWALSMIGMSLGLHRYFAHRAFKTSETMRVILAILGCMGAQGPVVSWVAVHRRHHEYSDLPGDPHSPNPELLGEGIFGKG	145

Figure 1. *Cont.*

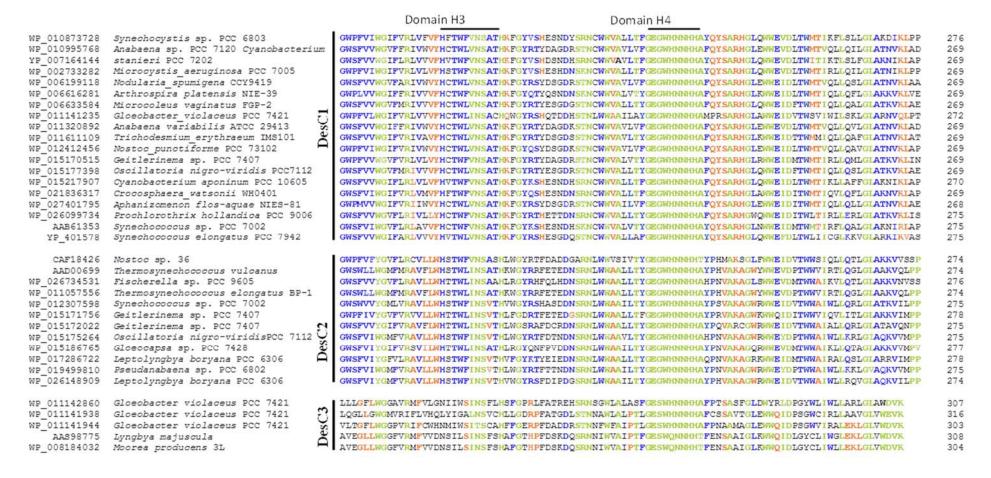**Figure 1.** Alignment of partial amino acid sequences of the acyl-lipid fatty acid Δ9-desaturases from different cyanobacteria. The desaturases are clustered into three types of enzymes, DesC1, DesC2, and DesC3, according to their amino acid and functional features. Four conservative histidine-containing domains are marked. Amino acids identical or similar in all three groups of the Δ9-desaturases are shown in **green**; amino acids identical in two groups of desaturases are shown in **blue**; amino acids, which are unique for one of the desaturase groups, are shown in **orange**.

2.2. Cyanobacteria of Group 2

Previously, cyanobacteria that produce only mono- and dienoic FAs were unknown [13]. Therefore, Group 2 contained cyanobacteria capable of producing trienoic α-linolenic acid, $18:3\Delta^{9,12,15}$ (*Anabaena*, *Nostoc*, *Gloeobacter violaceus*, *etc.*). Now we know a number of organisms that desaturate C_{18} and C_{16} FAs at positions Δ^9 and Δ^{12} to produce mono- and dienoic fatty acids.

Genomes of these organisms contain genes for Δ9- and Δ12-desaturases. These are, mainly, representatives of marine species, *Prochlorococcus* and *Synechococcus*. We propose to allocate these cyanobacteria into Group 2 (Table 2). The analysis of lipids and FA composition of these organisms is still limited and requires detailed studies. In some plant, fungi, protist, and animal species, FA desaturases may possess bifunctional activities; one enzyme may catalyze two reactions, for example, the formation of double bonds at Δ^{12} and ω^3 (Δ^{15}) positions [22,30]. Such bifunctional enzymes have not been yet reported in cyanobacteria. However, to confirm their absence, more experimental evidence is necessary on lipids and FAs for cyanobacteria of Group 2.

The freshwater filamentous *Prochlorothrix hollandica* differs from other cyanobacteria by the presence of light-harvesting chlorophyll *a/b* binding antenna and by the absence of phycobilins. *Prochlorothrix hollandica* is known as a C_{14}-rich organism, which contains 5% of 14:0 and 30% of $14:1\Delta^9$ in lipids [31]. *Prochlorothrix*, together with Δ9-desaturase, has the unique Δ4-desaturase activity and produces unusual $16:1\Delta^4$ (25%) and $16:2\Delta^{4,9}$ (10%) FAs [31]. The genetic data for the cyanobacterial Δ4-desaturase is still unavailable. Nevertheless, the presence of high amounts of $16:2\Delta^{4,9}$ (and the complete absence of 18:2 FAs) should place *Prochlorothrix hollandica* to a special position in Group 2 of cyanobacteria, which are capable of synthesizing the dienoic FAs.

2.3. Cyanobacteria of Group 3

According to previously proposed classification, the cyanobacterial strains that synthesize trienoic α-linolenic acid, $18:3\Delta^{9,12,15}$ were assigned to Group 2. These organisms have three distinct FA desaturase activities: Δ9-, Δ12- and Δ15-desaturases. Organisms of a former Group 3 also have three distinct desaturases, but, instead of Δ15, they introduce a third double bond at position Δ^6 and produce trienoic γ-linolenic acid, $18:3\Delta^{6,9,12}$, as a final product of desaturation.

We propose to combine all organisms that produce trienoic FAs as the final products of desaturation into Group 3, which will be divided into two subgroups—Group 3α and Group 3γ—according to the final product of desaturation—α- or γ-linolenic acids (Table 2).

Cyanobacteria that belong to a newly proposed Group 3α produce α-linolenic acid, $18:3\Delta^{9,12,15}$. These species (*Leptolyngbya boryana* (formerly, *Plectonema boryanum*), *Gloeobacter violaceus*, *Anabaena* sp., *Synechococcus* sp. PCC 7002, *Trichodesmium erythraeum*, some *Nostoc* species) are characterized both genetically and biochemically.

Genome sequencing of these species confirmed the presence of genes for the specific Δ9-, Δ12-, and Δ15-desaturases [24,32–34]. Lipid and FA analysis revealed the presence of 16:0, $16:1\Delta^9$, 18:0, $18:1\Delta^9$, $18:2\Delta^{9,12}$, and $18:3\Delta^{9,12,15}$ FAs [13,23,29,35–38].

The presence of a single strain of marine *Synechococcus* in this group (namely, *Synechococcus* sp. PCC 7002) raises a question about possible diversity of this genus in terms of FA composition. Table 2

clearly demonstrates that freshwater *Synechococcus* strains synthesize monoenoic FAs and belong to Group 1, whereas marine *Synechococcus* strains synthesize dienoic FAs and belong to Group 2. Alternatively, it may raise a question about the correct assignment of a strain PCC 7002 to a genus of *Synechococcus*.

Cyanobacteria of Group 3γ are capable of synthesizing the γ-linolenic acid, $18:3\Delta^{6,9,12}$. These organisms have three distinct FA desaturase activities: Δ6-, Δ9- and Δ12-desaturases. These cyanobacteria are represented by species of filamentous *Arthrospira* (*Spirulina*), unicellular *Synechocystis* sp. PCC 6714, and *Synechocystis* sp. IPPAS B-274.

The genomic and biochemical data for *Arthrospira* [29,39–41] and *Synechocystis* sp. PCC 6714 [13,42] are available, which support the positioning of these strains to Group 3. *Synechocystis* strains PCC 6714 and PCC 6803 are thought to be closely related species [42]. However, unlike *Synechocystis* PCC 6803 (Group 4, see below), *Synechocystis* sp. PCC 6714 lacks a gene for the ω3(Δ15)-desaturase [42], and it cannot synthesize α-linolenic and/or stearidonic acid.

2.4. Cyanobacteria of Group 4

Cyanobacteria of Group 4 have four acyl-lipid fatty acid desaturases and they can synthesize tetraenoic stearidonic acid, $18:4\Delta^{6,9,12,15}$, from C_{18} saturated stearic acid. In a model strain, freshwater unicellular *Synechocystis* sp. PCC 6803, synthesis of α-linolenic and stearidonic acids is temperature-dependent and occurs only at low temperatures (15–25 °C) [43]. Therefore, biochemical analysis cannot reveal 18:3α and 18:4 FAs in cells grown at optimal temperatures (30–36 °C). Genome sequencing [44] together with gene expression analysis [45] demonstrated the presence and expression of genes for Δ6-, Δ9-, Δ12-, and Δ15(ω3)-desaturases in *Synechocystis* sp. PCC 6803. And besides, the gene for Δ15(ω3)-desaturase was active only at low temperatures [45].

Similarly, genome sequence analysis of the marine filamentous cyanobacteria *Nodularia spumigena* and *Lyngbya* sp. PCC 8106 revealed the presence of genes for Δ6-, Δ9-, Δ12-, and Δ15(ω3)-desaturases [17]. Thus, these cyanobacteria would potentially produce α-linolenic, γ-linolenic, and stearidonic acids, the latter as a final product of desaturation.

Solid biochemical evidence is available for freshwater filamentous *Tolypothrix* species that confirms the presence of tri- and tetraenoic C_{18} FAs [12,13,29]. Recent lipid analysis of two strains, *Tolypothrix tenuis* and *Tolypothrix distorta* revealed previously undetected positional isomer of stearidonic acid, $18:4\Delta^{3,6,9,12}$ [46]. This so-called, γ-stearidonic acid was present in cells nearly in trace amounts. If this data is confirmed, it would be challenging to find a new cyanobacterial desaturase with Δ^3 specificity. The complete genomic sequence of *Tolypothrix* may clarify whether a fifth, yet unknown, desaturase exists in cyanobacteria, or a double bond at position Δ^3 is formed due to non-specific activity of Δ15(ω3)-desaturase on C_{16} FA, which is further elongated to C_{18}.

2.5. Adaptive and Taxonomic Impact of Cyanobacterial Fatty Acid Composition

Cyanobacteria are characterized by rather limited set of FAs in their lipids: C_{14}-C_{18} FAs with 1–4 double bonds. However, they have diverse phenotypes, and they inhabit very diverse environments, which, in many cases, are highly extreme. Fatty acid composition can be used to characterize different species of cyanobacteria, although the exact taxonomic meaning of FA composition is not completely

understood. The organization or complexity of cyanobacterial cells (unicellular or filamentous) does not correlate with FA composition. A number of double bonds in FAs correlates instead with temperature of the environment. Thermophilic unicellular species usually have monoenoic FAs, whereas mesophilic or psychrophilic unicellular species produce polyunsaturated FAs, which help them to survive at low temperatures by adjusting the membrane fluidity [7]. Thermophilic filamentous species adjust the membrane fluidity by the inhibition of 16:0 acid elongation and by enhancement of the monoenoic 16:1 acid synthesis [29]. In mesophilic species, both mechanisms—accumulation of 16:1 and desaturation—may be active. In mesophilic filamentous *Anabaena variabilis*, a drop in temperature leads to accumulation of C_{16} in the dark, and to formation of polyunsaturated FAs (mainly, C_{18}) in the light [29,47].

Fatty acid composition may be used to clarify the taxonomic position of a certain cyanobacterial strain. Thus, for example, it is rather surprising to find the representatives of genus *Synechococcus* (*Synechococcus* sp. PCC 7002) in diverse Groups 1, 2, and 3. Several authors noticed that the strains comprising the *Synechococcus* genus seem to be polyphyletic, and they suggested that this genus should be separated into different groups [48,49].

In general, the taxonomy of cyanobacteria is complicated and unclear [19,50]. The easiest and mostly used profiling technique employs the 16S rRNA gene sequence clustering. However, this simplified approach often leads to false assignments of strains and incorrect annotations. A more promising way to classify cyanobacterial strains is a polyphasic approach, which takes into consideration molecular, morphological, biochemical, and physiological characteristics of individual cultures and strains [51,52]. Recent developments in genome sequencing techniques provide a powerful tool for genetic profiling of cyanobacterial strains implying that sequence annotations are accurate. In such a polyphasic approach, the fatty acid composition is still a valuable marker to the cyanobacterial taxonomy.

3. Conclusions

The taxonomic system of cyanobacteria is developing according to combined multiple markers, including molecular, biochemical, ultrastructural, phenotypic and ecological data. The previously proposed system of biochemical classification of cyanobacteria according to their FA composition [11–13] is also changing. Here, we propose an update to this system according to newly available genomic and biochemical data. The basis of the system remains unchanged: cyanobacteria are grouped according the number of double bonds in their FAs. The major improvements are as follows. (1) The replacement of organisms in a previous "Group 2" with a new "Group 2" represented mainly by marine unicellular species, which are characterized by the presence of Δ9- and Δ12-desaturases and are capable of producing 16:2 or 18:2 FAs as the final product of FA desaturation. (2) Organisms previously assigned to Group 2 are transferred into Group 3, Subgroup 3α. Strains in this group are characterized by the presence of Δ9-, Δ12-, and Δ15(ω3)-desaturases, and they synthesize 18:3 α-linolenic acid as a final product of FA desaturation. (3) Organisms of the former "Group 3" (they have Δ6-, Δ9-, and Δ12-desaturases, and they synthesize 18:3 γ-linolenic acid) remain in Group 3, but placed into Subgroup 3γ. Group 1 (includes organisms that have Δ9-desaturase(s) and produce only monounsaturated FAs), and Group 4 (organisms with four FA desaturases, namely Δ6-, Δ9-, Δ12-, and Δ15(ω3)-desaturases, which may synthesize 18:4 stearidonic acid) remain unchanged.

These changes in FA-based classification system are necessary in order to adjust and synchronize biochemical, physiological and genomic data, which may help to establish an adequate comprehensive taxonomic system for cyanobacteria in the future.

Author Contributions

Kirill S. Mironov analyzed amino acid and genomic sequence data; Dmitry A. Los designed research and wrote the manuscript. Both authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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