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CHEMOTAXONOMY OF CYANOBACTERIA

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Abstract—The fatty acid-based classification system divides cyanobacteria into four groups. These groups include strains that contain C_{18} fatty acids with three, four or no double bonds. The fatty acid composition of several cyanobacterial strains that we have analysed suggests the existence of a fifth group that consists of C_{18} polyunsaturated fatty acids with no more than two double bonds.

INTRODUCTION

Differences in the fatty acid composition of cyanobacteria led Kenyon [1] and Kenyon *et al.* [2] to suggest criteria for their classification into four groups. The first group include cyanobacterial strains devoid of polyunsaturated fatty acids (PUFA), containing only saturated and mono-unsaturated fatty acids. The second and third groups consist of strains containing either α-linolenic acid (ALA, 18:3ω3) or γ-linolenic acid (GLA, 18:3ω6), respectively, while strains belonging to group four also contain octadecatetraenoic acid (18:4ω3). These findings were later confirmed by Murata *et al.* [3]. Kenyon *et al.* [2] have further claimed that unicellular strains belong to the first and third groups, while filamentous strains were divided between the second, third and fourth groups. However, Murata *et al.* [3] demonstrated that filamentous and unicellular strains were distributed amongst the four groups.

Cohen *et al.* [4] and Cohen and Vonshak [5], examining the fatty acid composition of several *Spirulina* strains, have shown that some of them typically contain GLA. On the contrary, other strains, characterized by a smaller cell size did not contain any GLA, but were rich in other fatty acids, such as ALA or 16:2. In spite of the similar coiling of trichomes, these two groups of strains actually belong to two separate genera, *Arthrospira* and *Spirulina*, respectively, improperly unified by Geitler's revision [6] to a single genus, *Spirulina*. After the official settlement of the taxonomic position of the genus *Arthrospira* made by Castenholz [7], so as to designate the large-sized alkalophilic species commercially cultivated for nutritional purposes (e.g. *A. platensis*), the designation of *Spirulina* should be restricted to the small-sized species, like *S. subsalsa*.

In the present work, we demonstrate the existence of some cyanobacteria belonging to the species *S. subsalsa* containing C_{18} PUFA with not more than two double bonds, thus requiring modification of the Kenyon–Murata classification system.

RESULTS AND DISCUSSION

Polar lipids of cyanobacteria contain C_{18} and C_{16} fatty acids which are esterified to the *sn*-1 and *sn*-2 positions, respectively, of the glycerol moiety. Kenyon *et al.* [2] classified cyanobacteria into four groups according to the mode of desaturation of fatty acids. Murata *et al.* [3] further elaborated this classification system and determined that the basis for classification is the number of double bonds and their position in the C_{18} fatty acids at the *sn*-1 position of the polar lipids. The first group includes cyanobacterial strains devoid of PUFA, which contain only saturated and mono-unsaturated fatty acids, such as palmitoleic (16:1) and oleic acids (18:1). Groups 2 and 3 consist of strains containing ALA and GLA, respectively. Strains belonging to group 4 contain both GLA and ALA as well as 18:4ω3 in the *sn*-1 position of their polar lipids. However, this classification system excludes the occurrence of cyanobacterial strains containing PUFA with only two double bonds, such as 16:2 or 18:2.

We have analysed the fatty acid composition of several *S. subsalsa* strains and evaluated the effect of growth temperature on the degree of fatty acid unsaturation (Table 1). Strain I2 contained 18:2 (20.8% of fatty acids) and 16:2 (1.1%), but no GLA, ALA or 18:4 were detected. Arguably, the lack of C_{18} PUFA with three or more double bonds in this strain could have resulted from the relatively high temperature (30°) at which it was cultivated, since it is known that the degree of unsaturation decreases with increasing growth temperature. We have thus also studied the fatty acid composition of this strain

Table 1. Fatty acid composition (% total fatty acids) of *Spirulina*-like cyanobacterial strains

Fatty acid composition	I2 (30°)	I2 (22°)	Sub [4] (30°)	1som6 (30°)	3F (30°)
16:0	26.8	22.6	49.2	33.0	41.7
16:1ω9	3.1	2.2		0.3	1.6
16:1ω7	38.6	43.5	35.0*	26.1	0.4
16:1ω5†	1.4	1.3		1.7	1.7
16:2	1.1	1.1		0.3	
16:3‡	0.4	0.3		0.5	—
16:3‡	0.9	0.9		0.4	0.5
16:4‡	0.5	0.7		0.2	
18:0	0.9	0.4	1.7	1.9	1.9
18:1ω9	4.8	1.6	1.0*	16.6	2.3
18:1ω7	0.8	0.6		1.9	2.2
18:2ω6	20.8	24.8	13.1	16.6	4.2
18:3ω3				0.4	43.5
18:3‡				1.8	3.8
Medium	Zarrouk [16]	Zarrouk [16]	ESW§	ESW	ESW

*Total isomers.
†Tentative assignment of double bond position.
‡Double bond positions not determined.
§Enriched seawater.

at 22° (Table 1). In agreement with Murata *et al.* [3], we found that the proportion of 18:2 increased from 20.8 to 24.8% and that of 16:2 increased from 1.1 to 1.3%. However, the changes were only quantitative and not even trace levels of either 18:3 or 18:4 were observed. Similarly, Cohen *et al.* [4] have shown that *S. subsalsa* contained 13.1% of 18:2 as its sole PUFA. Strain 1Som6 contained, at 30°, 18:2 (16.6%) as its main PUFA. However, small (< 0.5%) amounts of 16:3, 16:4 and 18:3ω3 were also present. When the growth temperature was reduced to 22°, the proportion of ALA increased to 5.4% (data not shown). Strain 3F had a very high proportion of ALA (43.5%) and no GLA. Both 1som6 and 3F contained relatively low levels (1.8 and 3.8%, respectively) of an unidentified C_{18} fatty acid, which was chromatographically different from both GLA and ALA. The occurrence of ALA places these strains in group 3. The absence of GLA from these strains further supports the distinction between these cyanobacteria and those traditionally used for nutritional purposes, e.g. *A. platensis*, commercially known as Spirulina.

We have fractionated the lipid extract of strain I2 into various lipid classes using 2-D TLC (Table 2). In monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), the major fatty acid was 16:1ω7, which amounted to *ca* 50% of the total fatty acids. The proportions of 16:0 and 18:2 were in the 15–20% range. Low levels (< 2%) of a 16:1 fatty acid tentatively identified as 16:1ω5 were also detected. The fatty acid compositions of the minor lipids, sulphoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), were significantly different. The major fatty acid was 16:0 (46–53%) and the proportions of 16:1ω7 were rather low. However, these lipids contained 4.5 and 16.4% of 16:1ω9, respectively. Similar to other cyanobacteria, PG contained no 16:1t(ω13). In edible strains of '*Spirulina*', the major C_{16} fatty acid in polar lipids is 16:0 and only a small percentage of various 16:1 isomers are found [4]. Murata *et al.* [3] have also studied the fatty acid composition of the individual polar lipids in representative cyanobacteria of each of the four groups. According to this study, an undisclosed isomer of a 16:1 fatty acid occurred as a major component only in polar lipids of group 1, and in MGDG and DGDG of group 2. In contrast, we found that 16:1ω7 is the major fatty acid in both MGDG and DGDG of strain I2, while 16:1ω9 is the dominant 16:1 isomer in SQDG, especially so in PG. To the best of our knowledge, the occurrence of distinct 16:1 isomers in polar lipids of cyanobacteria has not been reported before.

Based on our data, and by analogy with the composition of molecular species of the polar lipids of cyanobacteria as elucidated by Murata *et al.* [3], we suggest that the major molecular species of the polar lipids in strain I2 are probably 18:2/16:1ω7 and 16:1ω7/16:0 in MGDG and DGDG, 18:2/16:0 and 18:1/16:0 in SQDG and 18:2/16:0 and 16:1ω9/16:0 in PG.

The current classification system of cyanobacteria, based on the degrees of fatty acid desaturation, cannot accommodate strains such as I2 and *sub*. We thus suggest the existence of a fifth group of cyanobacteria. Strains belonging to this group contain 18:2 as their only C_{18} PUFA. Based on the hierarchy set by Murata *et al.* [3], the new group should be positioned between groups 1 and 2. Strains I2 and *subsalsa* belong to this new group.

Theoretically, there could be a sixth group of cyanobacterial strains containing both GLA and ALA, but no 18:4. However, Wada *et al.* [8] have shown that

Table 2. Fatty acid composition (% total fatty acids) of the polar lipids of strain I2

Lipid	Fatty acid composition							
	16:0	16:1ω9	16:1ω7	16:1ω5*	18:0	18:1ω9	18:1ω7	18:2ω6
MGDG	15.4		50.8	1.9	8.7	4.8	1.3	17.1
DGDG	17.1		46.4	1.8	6.8	6.0	1.4	20.5
SQDG	46.2	4.5	2.6		4.7	11.4	1.7	28.9
PG	53.3	16.4	1.8		5.5	7.9	1.7	13.4

*Tentative assignment of double bond position

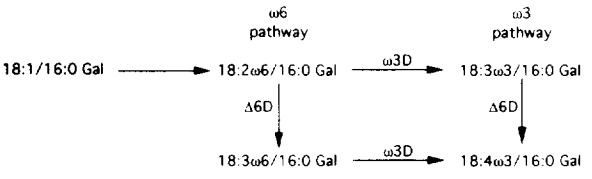

Fig. 1. Pathways of fatty acid desaturation in galactolipids of cyanobacterial strains containing 18:4. Δ6D Δ6 desaturase; ω3D — ω3 desaturase; Gal Galactose residue.

mutants of the cyanobacterium, *Synechocystis* PCC6803, deficient in either the ω3 desaturase or the Δ6 desaturase, did not produce 18:4. It follows that ω3 and Δ6 desaturases, the enzymes that desaturate 18:2 to ALA and GLA, respectively, can further desaturate these fatty acids to 18:4 (Fig. 1). The production of 18:4 is thus the consequence of the coexistence of these two enzymes. Therefore, the occurrence of cyanobacterial strains containing ALA and GLA, but no 18:4, is highly unlikely. The two pathways that start from 18:2 and ALA could be the early ancestors of the ω6 and ω3 pathways in eukaryotic algae, which presumably result in the production of arachidonic acid (20:4ω6) and eicosapentaenoic acid (20:5ω3), respectively. In cyanobacteria, there is a cross-over between these pathways, and 18:3ω6 can thus be converted into 18:4ω3. There are some indications that similar cross-overs exist also in eukaryotic algae [9–11].

EXPERIMENTAL

Organisms. Cyanobacterial strain 1Som6 was isolated from hypersaline water in a coastal lagoon in Somali [12]. Strain 3F was isolated from brackish water collected from a 10 m depth in Lake Faro (Messina, Italy) [13]. Strain I2 was obtained from Dr A. Vonshak and was isolated from alkaline water in China [14]. Because of the trichome shape, as a tightly coiled helix, gliding motility consisting of a cork screw motion and morphological parameters (trichome width 1–2 μm and coil width 3–5 μm) the strains were assigned to *S. subsalsa* Oersted [15]. Strains 1Som6 and I2 showed typical blue–green pigmentation, while strain 3F showed a red pigmentation, owing to its high phycoerythrin cell content. Though the cultures were not axenic, bacterial contamination was negligible in strain I2 and was less than 10% of the biomass in the other two strains.

Cultivation. S. subsalsa I2 was cultivated on Zarrouk medium [16]. Strains 1Som6 and 3F were grown at pH 7.8 in enriched seawater [12]. Cells were grown in an incubator at 30° and 22°, in an atmosphere of CO_2-enriched air (5%, v/v) under continuous irradiation of 70 μmol photons $m^{-2} s^{-1}$.

Fatty acid analysis. Freeze-dried cells were transmethylated with MeOH–AcCOCl as described previously [17]. GC analysis was performed on a Supelcowax 10 fused-silica capillary column (30 m × 0.32 mm) at 195° (FID, inj. and detector temp. 230°, split ratio 1:100). Peak areas were measured using an integrator. Fatty acid Me esters were identified by co-chromatography with authentic standards (Sigma) and by comparison of their equivalent chain lengths. In cases where unidentified peaks were obtained, fatty acid analysis was repeated on a lipid extract as follows. Biomass was extracted with $CHCl_3$, MeOH and H_2O according to ref. [18]. The lipid extract was hydrolysed with NaOH in MeOH and any non-hydrolysable lipid was washed with hexane. The aq. phase was acidified with HCl, extracted with hexane and methylated as detailed above. The data shown represent mean values with a range of < 3% for major (> 10% of fatty acids) peaks and 10% for minor peaks.

Lipid fractionation. Sepn into neutral lipid, galactolipids (GL) and phospholipids frs was performed using a silica gel cartridge (Sep-Pak). Individual frs were eluted successively with $CHCl_3$, Me_2CO and MeOH, respectively. The GL fr. was further resolved by TLC using $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4:2:2:1).

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REFERENCES

1. Kenyon, C. N. (1972) *J. Bacteriol.* **109**, 827.
2. Kenyon, C. N., Rippka, R. and Stanier, E. Y. (1972) *Arch. Microbiol.* **83**, 216.

3. Murata, N., Wada, H. and Gombos, Z. (1992) *Plant Cell Physiol.* **33**, 933.
4. Cohen, Z., Vonshak, A. and Richmond, A. R. (1987) *Phytochemistry* **26**, 2255.
5. Cohen, Z. and Vonshak, A. (1991) *Phytochemistry* **30**, 205.
6. Geitler, L. (1932) in *Rabenhorst's Kryptogamenflora von Deutschland, Oesterreich und der Schweiz* (Kolkwitz, R., ed.), Vol. 14. Akademische Verlagsgesellschaft, Leipzig, Germany.
7. Castenholz, R. W. (1989) in *Bergey's Manual of Systematic Bacteriology* (Stanley, J. T., Bryant, M. P., Pfenning, N. and Holt, J. G., eds), Vol. 3, p. 1771. William and Wilkins, Baltimore, MD.
8. Wada, H. Gombos, Z., Sakamoto, T., Higashi, S., Los, D. A., Heinz, E., Schmidt, H., Nishida, I. and Murata, N. (1993) in *Biochemistry and Molecular Biology of Membranes and Storage Lipids* (Murata, N. and Somerville, C. R., eds). The American Society of Plant Physiology, Rockville, MD.
9. Cohen, Z., Didi, S. and Heimer, Y. M. (1992) *Plant Physiol.* **98**, 569.
10. Schneider, J. C. and Roessler, P. (1994) *J. Phycol.* **30**, 594.
11. Arao, T. and Yamada, M. (1994) *Phytochemistry* **35**, 1177.
12. Tredici M. R., Margheri, M. C., De Philippis, R., Bocci, F. and Materassi, R. (1988) *Int. J. Solar Energy* **6**, 235.
13. Tomaselli, L., Margheri, M. C. and Sacchi, A. (1955) *Mar. Microb. Food Webs* **9** (in press).
14. Qian, K., Sato, G. H., Zhao, V. and Shinohara, K. (1994) in *Genetic Engineering. Principles and Methods* (Setlow, J. K. and Hollander, A., eds), Vol. 6, p. 309. Plenum Press, New York.
15. Desikachary, T. V. (1959) *Cyanophyta.* Indian Council of Agricultural Research, New Delhi.
16. Zarrouk, C. (1966) Ph. D. Thesis. University of Paris, France.
17. Cohen, Z., Vonshak, A. and Richmond, A. R. (1988) *J. Phycol.* **24**, 328.
18. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.