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FATTY ACID COMPOSITION OF *SPIRULINA* AND *SPIRULINA*-LIKE CYANOBACTERIA IN RELATION TO THEIR CHEMOTAXONOMY

ZVI COHEN* and AVIGAD VONSHAK

Laboratory for Microalgal Biotechnology, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede-Boker Campus 84990, Israel

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Abstract—The fatty acid composition of several *Spirulina*-like cyanobacteria strains was investigated. While, on the basis of morphology alone, these strains could not be distinguished from *Spirulina*, their fatty acid composition demonstrated a different pattern having large amounts of 18:3 (9, 12, 15), 16:1 or 16:2, but small quantitites of γ-linolenic acid. On the basis of these findings, it is suggested that fatty acid composition might be utilized for the classification of *Spirulina* strains.

INTRODUCTION

The cyanobacterium *Spirulina* is one of the few commercially cultivated microalgae used primarily as a health food. *Spirulina* is also one of the few organisms known to have a high content of γ-linolenic acid [GLA, 18:3 (6, 9, 12)] [1–4]. This fatty acid is known to be of value in the treatment of various diseases [5–9].

Although a few commercial sites for production of *Spirulina* are operated, very little is known about the strains used for commercial production. Because the classification system used for *Spirulina* is essentially morphological and mainly based on its spiral shape, it is difficult to distinguish between any strains used. The morphology of the filaments is highly dependent on growth conditions and environmental factors [10] and under certain conditions the filaments may even lose their spiral shape [11]. Fatty acid distribution is one of the tools available for taxonomic classification of microalgae but its use is generally limited to the level of classes and orders but not for a genus. Kenyon *et al.* used fatty acid composition as a criterion for dividing cyanobacteria into several subgroups [12] and claimed the existence of two strains of *Spirulina*, one containing α-linolenic acid [18:3(9, 12, 15), ALA] and no GLA, the other one being devoid of both fatty acids. Based on these data, the validity of the commonly used classification system has already been questioned by Wood [13].

In a previous study, we have shown [14] that 18 strains of *Spirulina* obtained from culture collections, or isolated from their natural habitat, had the same fatty acid composition, the major fatty acids being 16:0, 16:1, 18:0, 18:1, 18:2 and GLA. The proportion of GLA is quite high, generally ranging in % of total fatty acids between 19.3 and 31.7% at 35°. Only one strain (2340) had a significantly lower proportion (8%) which increased however to 23% at lower temperature (25°). All of these strains were cultivated on the standard alkaline *Spirulina* medium (Zarrouk's medium, pH 9.8). These findings were in agreement with those described in most other reports [1, 15–17]. The great commercial interest in *Spirulina* and the need for applying modern techniques of strain selection makes it important to set standards for classification other than those based on morphology. In this paper, we provide evidence suggesting that the fatty acid composition of *Spirulina* may be used as a basis for characterization.

RESULTS AND DISCUSSION

We studied the fatty acid composition of six strains, originally characterized as *Spirulina*, based on their morphological appearance and pigment composition, yet which did not grow on alkaline medium. These strains were spiral shaped, filamentous with no heterocystous cells, similar to the *Spirulina* strains previously studied. Four strains (Th 11, Th 21, Th 30, Sh) displayed similar characteristics in their fatty acid composition (Table 1). Although no GLA was found in these strains they contained high proportions of ALA. Also, the proportion of palmitoleic acid 16:1 was unusually high compared with GLA containing *Spirulina* strains ranging from 13.8 to 32.3%. The amount of 16:1 in alkaline strains was in the range 0.5–4.4% (% total fatty acids).

Strain LB 2179 had a higher proportion of ALA (35.3%) and contained a significant proportion of another fatty acid (16:2) found only in trace amounts in alkaline strains. Only one freshwater strain (N 27), contained GLA (2.6%). However, this strain is unique in its high 16:1 and 16:2 contents, 18.6 and 19.2%, respectively.

In a previous study we found [14] another strain, *S. subsalsa*, which did not grow on the alkaline medium but could be cultivated on an artificial sea water medium. Its fatty acid composition was significantly different. No trace of GLA was found and the proportion of 16:1 was 35.0% as compared with 0.5–4.4% in the other strains.

It appears that several cyanobacteria, although morphologically indistinguishable from *Spirulina*, display

*Author to whom correspondence should be addressed.

Table 1. Fatty acid composition of *Spirulina*-like strains

Strain	Medium	Fatty acid composition*									
		14:0	16:0	16:1	16:2	16:3	18:0	18:1	18:2	ALA	GLA
Th 11	BG 11	1.4	41.2	13.8	—	1.7	2.6	8.9	18.1	12.3	—
Th 21	BG 11	1.8	40.6	21.2	—	0.3	1.0	3.1	13.2	18.8	—
Th 30	BG 11	2.5	40.4	22.3	—	0.5	1.0	3.4	13.7	16.2	—
Sh	BG 11	0.6	33.1	16.8	0.2	1.2	1.4	5.8	18.7	20.5	—
LB 2179	BG 11	2.2	31.2	7.6	9.5	0.8	1.7	4.9	6.9	35.3	—
N 27	BG 11	2.9	47.0	18.6	19.2	2.3	0.6	1.8	5.0	—	2.6

*Wt per cent of total fatty acids.
ALA = α-linolenic acid.
GLA = γ-linolenic acid.

patterns of fatty acid distribution which are different from *Spirulina* both qualitatively and quantitatively. These dissimilarities appear to be significant enough as to exclude from classification in the genus *Spirulina*. This argument is further strengthened by the finding that these strains will generally not grow in the typical alkaline *Spirulina* medium. One may argue that the differences in fatty acid compositions are just manifestations of the different cultivation conditions resulting from the differences of pH and salinity between the various media. However, the fact that the freshwater strain N 27 contains GLA and not ALA suggests otherwise. Moreover, the differences between the enzymatic systems necessary to generate GLA and ALA are considerable and only rarely can both GLA and ALA be found in the same organism. These results suggest that cyanobacteria unable to grow in an alkaline medium are probably unrelated to *Spirulina*. We propose that in addition to the already existing morphological criteria for *Spirulina*, the fatty acid composition should be used as an additional criterion for the characterization of the genus. Authentic *spirulina* strains display a significant proportion of GLA, contain no ALA, and probably also contain no more than a low content of 16:1 (< 10%) and a very low content of 16:2 (< 0.1%).

EXPERIMENTAL

Organisms. Cyanobacterial strains Th-11, Th-21 and Th-30 were isolated from standing water during the dry season in northeast Thailand. Strain Sh was isolated from fish ponds in the north of Israel by Prof. M. Shilo and coworkers (Hebrew University, Jerusalem, Israel). Strains LB 2179 was obtained from the University of Texas Culture Collection and strain N 27 from the Microbial Culture, The National Institute for Environmental Studies, Japan. These strains were grown on BG-11 medium. Algal cultures were unialgal. Bacterial counts did not exceed 50 viable counts of bacteria per ml, as measured by plating samples of the cultures on nutrient agar containing *Spirulina* medium.

Culture conditions. Cultures were cultivated at 35° as previously described [14].

Fatty acid analysis. Freeze-dried samples of algal biomass were transmethylated with MeOH–AcCl as previously described [18]. Heptadecanoic acid was added as int. standard. Gas chromatographic analysis was performed on a Supelcowax 10 fused silica capillary column (30 m, 0.32 mm) at 200° (FID, inj. and flame ionization detector temps 230°, split ratio 1:100). Fatty acid Me esters were identified by co-chromatography with authentic standards (Sigma) and by GC-MS using a Carbowax capillary column (30 m). CI spectra were obtained at 250 eV with *iso*butane as reactant gas. Fatty acid contents were determined by comparing their peak areas after integration with that of the int. standard. Data shown represent mean values (with a range of less than 3% for major peaks and 8% for minor peaks) of at least two independent samples, each analysed in duplicate.

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