

Fatty Acids Composition Analysis from Laboratory Marine Cyanobacterial Culture

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Abstract

Seven marine cyanobacteria isolated from mangrove environment, purified, cultured in standard culture medium and analysed fatty acid composition. Among saturated fatty acids the palmitic acid found to be high percentage in all species. Capric acid obtained only in *Synechocystis salina*, pentadecanoic acid detected in *Oscillatoria salina* and *O. willei*. In *Oscillatoria cartiana* was the lowest percentage of saturated fatty acid (74.81 %) and highest percentage of unsaturated fatty acid (25.19 %). In *Synechocystis salina* was the highest percentage of saturated fatty acid (86.43 %) and lowest percentage of unsaturated fatty acid (13.57 %). The range of myristic acid varied from (1.41 - 2.75 %) in *Synechocystis salina* and *Oscillatoria willei*. The percentage of palmitic acid varied (6.67 - 13.1 %) in *Synechocystis salina* and *Phormidium fragile*. Stearic acid percentage ranged from (2.05 - 4.13 %) in *Oscillatoria willei* and *Phormidium tenue*. Most of the saturated and unsaturated fatty acids were statistically significant at 1% level.

Keywords: Marine Cyanobacteria; Myristic Acid; Palmitic Acid; Stearic Acid; Saturated Fatty Acid; Unsaturated Fatty Acid

Introduction

Recent pharmaceutical interest in unsaturated fatty acids has triggered the search for source of these valuable compounds from marine cyanobacteria. Monounsaturated fatty acids such as palmitoleic acid (16:1), undecylenic acid (11:1) and tridecanoic acid (13:1) have the potential for preventing several diseases [1]. Fatty acids play an important role in human metabolic pathways, particularly as specific precursors for prostaglandin E1 [2]. The γ -linolenic acid is recognized as a promising therapeutic agent for numerous health disorders acting as a precursor for prostaglandin E1, an important compound necessary for reducing inflammation and in treatment of heart disease, Parkinson disease, multiple sclerosis, plasma cholesterol levels, dermatitis, diabetes, and pre-menstrual syndrome [3-6]. The PUFA play an important role in regulating cell membrane properties and serve as precursors for important animal hormones and are found to be critical in maintaining high growth, survival and reproductive rates and hence play an important role in the aquaculture studies [7]. Cyanobacteria are rich in essential fatty acids such as C₁₈ linoleic (18:2 ω 6) and γ -linolenic (18:3 ω 3) acids and their C₂₀ derivatives, eicosapentaenoic acids (20:5 ω 3) and arachidonic acid (20:4 ω 6). These fatty acids are essential

components of the diet of human and animals and are becoming important feed additives in aquaculture [8]. Some of the filamentous cyanobacteria have large quantities (25 - 60 %) of polyunsaturated fatty acids [9-12] reported that *Spirulina platensis* accumulated large amount of gamma linolenic acid.

Materials and Methods

Lipid extracted by chloroform: methanol (2:1) solution. The extract was filtered by filter paper. Distilled water was added to remove the water soluble impurities. The filtrate was dried either under nitrogen or in a rotary evaporator. The fatty acids were analysed from known volume of lipid sample using saponification reagent, methylation reagent, extraction solvent and base wash solution by Gas chromatography. The sample mixed with saponification reagent and boiled for 30 minutes. Methylation reagent were mixed well and again boiled at 80°C for 20 minutes. Cooled down and mixed with extraction solvent. The sample tubes tightly closed and rotated end-over-end for 10 minutes. The aqueous lower phase was discarded and added base wash solution. The tube was rotated end-to-end for 5 minutes. 2/3 of the extract (upper phase) was transferred in to a GC vial. Methyl esters (8:0 - C24:20) was used as a standard. From the Retention Time (RT), the fatty acids of the sample were quantified by using peak area and the composition of individual fatty acid is expressed as percentage of total fatty acids [13].

Results

The composition of saturated fatty acids present in seven marine cyanobacteria is shown in Table 1. Capric acid (10:0) was observed only in *Synechocystis salina* even very low percentage (1.6 %) and it was statistically significant at 1% level. Five cyanobacterial species found Underonoic acid (11:0) with low percentage range (0.92 - 1.56 %) in *Oscillatoria willei* and *O. salina*. Lauric acid (12:0) range also low (1.67 - 2.86 %) in *Synechocystis salina* and *Oscillatoria willei* and found in all species except *O. cartiana*. All cyanobacteria species have Myristic acid (14:0) with low percentage range (1.41 - 2.75 %) in *Synechocystis salina* and *O. willei*. There were found only two species have only Pentadeiaxnoic acid (15:0) in *Oscillatoria salina* (0.65 %) and *O. willei* (0.86 %). Among the fatty acids the Palmitic acid has the highest percentage range from (6.67 - 13.1 %) in *Synechocystis salina* and *Phormidium fragile*. *Phormidium* sp. has highest percentage of Palmitic acid than *Oscillatoria* sp. and other species. Stearic acid was the second highest percentage fatty acid range from (2.15 - 4.13 %) in *Spirulina subsalsa* and *Phormidium tenue*.

No.	Fatty acids	<i>Synechocystis salina</i>	<i>Spirulina subsalsa</i>	<i>Oscillatoria cartiana</i>	<i>Oscillatoria salina</i>	<i>Oscillatoria willei</i>	<i>Phormidium fragile</i>	<i>Phormidium tenue</i>	'F' values
1	3OH (8:0)	-	1.59 ± 0.4	-	-	2.70 ± 0.2	-	-	92.16 **
2	Nanhanic acid (9:0)	-	1.38 ± 0.1	-	-	1.14 ± 0.1	-	-	319.87 **
3	Capric acid (10:0)	1.60 ± 0.2	-	-	-	-	-	-	44.90 **
4	Underonoic acid (11:0)	-	1.19 ± 0.1	1.23 ± 0.1	1.65 ± 0.2	0.92 ± 0.1	-	1.58 ± 0.2	19.79 **
5	ISO 3OH (11:0)	12.0 ± 0.7	11.3 ± 0.9	-	-	12.93 ± 0.8	18.03 ± 1.5	13.5 ± 0.9	132.49 **
6	Anteiso (11:0)	1.92 ± 0.1	-	2.99 ± 0.1	3.16 ± 0.3	-	-	-	323.50 **
7	2OH (11:0)	1.88 ± 0.2	-	2.46 ± 0.1	5.03 ± 0.7	-	-	-	88.69 **
8	Lauric acid (12:0)	1.67 ± 0.3	1.79 ± 0.3	-	1.84 ± 0.3	2.86 ± 0.6	2.45 ± 0.6	2.09 ± 0.2	3.29 *
9	ISO (12:0)	2.84 ± 0.4	2.91 ± 0.1	3.53 ± 0.3	5.58 ± 1.1	-	-	4.07 ± 0.2	17.19 **
10	Anteiso (12:0)	9.60 ± 0.9	-	11.2 ± 0.5	-	-	-	-	308.39 **
11	2OH (12:0)	0.88 ± 0.1	0.43 ± 0.1	-	-	0.73 ± 0.3	1.14 ± 0.1	0.78 ± 0.1	20.31 **
12	ISO 3OH (13:0)	-	-	-	-	-	-	0.91 ± 0.2	45.06 **
13	Anteiso (13:0)	5.11 ± 0.3	6.79 ± 0.1	7.11 ± 0.3	6.91 ± 0.8	4.97 ± 0.4	-	5.41 ± 0.7	78.15 **
14	Myristic acid (14:0)	1.41 ± 0.2	1.97 ± 0.1	2.20 ± 0.5	2.54 ± 0.2	2.75 ± 0.3	1.78 ± 0.2	2.18 ± 0.4	1.39 NS
15	ISO (14:0)	2.13 ± 0.4	1.21 ± 0.1	1.44 ± 0.4	1.59 ± 0.3	1.24 ± 0.2	1.47 ± 0.1	1.20 ± 0.2	5.73 **
16	Anteiso (14:0)	7.91 ± 1.3	9.90 ± 0.6	9.11 ± 0.5	9.49 ± 0.1	7.92 ± 0.8	10.4 ± 0.4	6.57 ± 1.3	3.16 *
17	Pentadeiaxnoic acid (15:0)	-	-	-	0.65 ± 0.1	0.86 ± 0.1	-	-	105.46 **
18	ISO 3OH (15:0)	1.01 ± 0.2	1.29 ± 0.1	-	1.52 ± 0.2	1.29 ± 0.1	1.44 ± 0.1	-	55.22 **
19	Anteiso (15:0)	6.56 ± 0.4	7.99 ± 0.5	8.25 ± 0.5	8.29 ± 0.4	6.28 ± 0.5	9.18 ± 0.3	5.65 ± 0.8	4.08 *
20	Unknown (15.665)	1.86 ± 0.3	1.85 ± 0.1	2.37 ± 0.2	2.39 ± 0.5	1.80 ± 0.1	2.81 ± 0.4	2.27 ± 0.5	0.60 NS

21	Palmitic acid (16:0)	6.67 ± 1.3	9.35 ± 1.9	9.97 ± 1.6	9.97 ± 1.5	10.5 ± 0.8	13.1 ± 0.5	12.6 ± 1.1	2.07 NS
22	Anteiso (16:0)	5.74 ± 0.5	7.15 ± 0.2	7.52 ± 0.5	7.90 ± 0.6	7.32 ± 1.4	9.86 ± 0.5	6.31 ± 1.2	1.02 NS
23	2OH (16:0)	2.03 ± 0.4	-	-	-	0.48 ± 0.1	0.54 ± 0.2	-	49.28 **
24	N alcohol (16:0)	0.95 ± 0.1	1.23 ± 0.1	-	-	-	-	-	137.24 **
25	10 methyl (16:0)	1.97 ± 0.9	1.95 ± 0.4	2.51 ± 0.1	2.72 ± 0.3	1.68 ± 0.4	3.40 ± 1.0	1.69 ± 0.1	0.61 NS
26	Anteiso (17:0)	4.73 ± 0.5	10.7 ± 1.3	-	6.54 ± 0.7	5.57 ± 0.9	-	5.21 ± 1.0	4.22 **
27	Stearic acid (18:0)	2.65 ± 0.4	2.15 ± 0.3	2.96 ± 0.3	2.65 ± 0.7	2.05 ± 0.3	4.06 ± 0.7	4.13 ± 0.6	5.07 **
28	Anteiso (19:0)	3.30 ± 0.8	-	-	-	-	-	-	35.26 **
29	Cyclo W8C (19:0)	-	-	-	-	-	1.38 ± 0.2	1.19 ± 0.1	108.09 **
	Total	86.43	84.11	74.81	80.42	75.99	81.04	77.35	

Table 1: Percentage composition of saturated fatty acids from 10 days old laboratory cyanobacterial culture (** - Significant at 1%; * - Significant at 5%; NS - Not significant).

The saturated fatty acids that exceed 5% of total saturated fatty acids were 11:0 ISO 3OH (12%), 12:0 Anteiso (9.6%), 13:0 Anteiso (5.11%), 14:0 Anteiso (7.91%), 15:0 Anteiso (6.56%), 16:0 Palmitic acid (6.67%), 16:0 Anteiso (5.74%) in *Spirulina subsalsa*. It was 11:0 ISO 3OH (11.3%), 13:0 Anteiso (6.79%), 14:0 Anteiso (9.9%), 15:0 Anteiso (7.99%), 16:0 Palmitic acid (9.35%), 16:0 Anteiso (7.15%) and 17:0 Anteiso (10.7%) in *Oscillatoria salina*. The saturated fatty acids that exceed 5% of total saturated fatty acids were 12:0 Anteiso (11.2%), 13:0 Anteiso (7.11%), 14:0 Anteiso (9.11%), 15:0 Anteiso (8.25%), 16:0 Palmitic acid (9.97%) and 16:0 Anteiso (7.52%) in *Synechocystis salina*. It was 11:0 2OH (5.03%), 12:0 ISO (5.58%), 13:0 Anteiso (6.91%), 14:0 Anteiso (9.49%), 15:0 Anteiso (8.29%), 16:0 Palmitic acid (9.97%), 16:0 Anteiso (7.9%) and 17:0 Anteiso (6.54) in *Phormidium tenue*. The saturated fatty acids that exceed 5% of total saturated fatty acids were 11:0 ISO 3OH (12.93%), 14:0 Anteiso (7.92%), 15:0 Anteiso (6.28%), 16:0 Palmitic acid (10.5%), 16:0 Anteiso (7.32%) and 17:0 Anteiso (5.57%) in *P. fragile*. It was 11:0 ISO 3OH (18.03%), 14:0 Anteiso (10.4%), 15:0 Anteiso (9.18%), 16:0 Palmitic acid (13.1%) and 16:0 Anteiso (9.86%) in *O. willei*. The saturated fatty acids that exceed 5% of total saturated fatty acids were 11:0 ISO 3OH (13.5%), 13:0 Anteiso (5.41%), 14:0 Anteiso (6.57%), 15:0 Anteiso (5.65%), 16:0 Palmitic acid (12.6%), 16:0 Anteiso (6.31%) and 17:0 Anteiso (5.21%) in *O. cortiana*. The values among the species were statistically significant in almost all the fatty acids except in Myristic acid (14:0), unknown 15.665, palmitic acid (16:0), anteiso (19:0) and 10 methyl (16:0) (Table 1).

The unsaturated fatty acid of cyanobacteria was studied in seven marine cyanobacteria species are shown Table 2. The

unsaturated fatty acid 3OH (12:1), W5C (16:1) and W11C (16:1) found only in *Oscillatoria cartiana* with less percentage 0.54, 3.42 and 2.84 % respectively. All the species found ISOG (15:1) unsaturated fatty acid with less percentage range from (0.93 - 1.67 %) in *Phormidium tenue* and *Oscillatoria cartiana*. The fatty acid W7C (16:1) observed in all cyanobacteria species with higher percentage range from (1.22 - 5.49 %) in *Spirulina subsalsa* and *Oscillatoria salina*. The fatty acid W5C (17:1) and Sum in 7 occurred only in *Phormidium tenue* with low percentage 0.54 and 2.40 % respectively. The unsaturated fatty acid ISOG (18:1) found in all species with less percentage range from (0.50 - 1.29 %) in *Oscillatoria willei* and *O. cartiana*. There were five species have W9C (18:1) unsaturated fatty acids with high percentage range (1.34 - 6.15 %) in *Oscillatoria salina* and *Phormidium tenue*. Among the different fatty acid, the Sum in 6 has highest percentage range from (4.63 - 7.21 %) in *Phormidium fragile* and *Spirulina subsalsa*.

The unsaturated fatty acids that exceed 4% of total unsaturated fatty acids were Sum in 6 (4.96%) in *Spirulina subsalsa*. It was Sum in 6 (7.21%) in *Oscillatoria salina*. The unsaturated fatty acids that exceed 4% of total unsaturated fatty acids were Sum in 6 (6.55%) in *Synechocystis salina*. It was Sum in 6 (6.76%) and 16:1 W7C (5.49%) in *Phormidium tenue*. The unsaturated fatty acids that exceed 4% of total unsaturated fatty acids were Sum in 6 (5.81%) and 18:1 W9C (4.7%) in *P. fragile*. The percentage was above 4% in *O. willei* 16:1 W7C (5.33%) 18:1 W9C (4.04%) and Sum in 6 (4.63%). The unsaturated fatty acids that exceed 4% of total unsaturated fatty acids were 18:1 W9C (6.15%) and Sum in 6 (4.96%) in *O. cortiana*. The values among the species were

statistically 1% significant in all fatty acids except in ISOG 15:1), ISOG (18:1) and Sum in feature 6 (Table 2).

No.	Fatty acids	<i>Synechocystis salina</i>	<i>Spirulina subsalsa</i>	<i>Oscillatoria cartiana</i>	<i>Oscillatoria salina</i>	<i>Oscillatoria willei</i>	<i>Phormidium fragile</i>	<i>Phormidium tenue</i>	'F' values
1	3OH (12:1)	-	-	0.54 ± 0.11	-	-	-	-	46.83 **
2	ISOG (15:1)	1.59 ± 0.55	1.64 ± 0.44	1.69 ± 0.19	1.67 ± 0.31	1.46 ± 0.27	1.59 ± 0.22	0.93 ± 0.23	1.38 **
3	ISOG (16:1)	0.99 ± 0.08	0.64 ± 0.10	-	-	-	-	-	180.78 **
4	W5C (16:1)	-	-	3.42 ± 0.52	-	-	-	-	14.23 **
5	W7C (16:1)	1.75 ± 0.66	1.22 ± 0.14	2.53 ± 0.27	5.49 ± 0.91	2.16 ± 0.10	5.33 ± 0.93	1.79 ± 0.26	7.54 **
6	W9C (16:1)	0.97 ± 0.17	-	-	-	2.68 ± 0.38	-	3.03 ± 0.17	115.04**
7	W11C (16:1)	-	-	2.84 ± 0.47	-	-	-	-	32.45**
8	W5C (17:1)	-	-	-	-	-	-	0.54 ± 0.08	81.77**
9	ISOG (18:1)	0.93 ± 0.15	0.99 ± 0.12	1.29 ± 0.21	0.52 ± 0.15	0.50 ± 0.13	1.25 ± 0.56	1.21 ± 0.16	2.80*
10	2OH (18:1)	-	1.48 ± 0.21	2.13 ± 0.21	1.96 ± 0.10	1.37 ± 0.47	2.12 ± 0.42	1.58 ± 0.22	9.21**
11	W5C (18:1)	1.40 ± 0.18	2.71 ± 0.43	-	1.84 ± 0.21	1.25 ± 0.10	-	-	58.84**
12	W9C (18:1)	-	-	4.20 ± 0.86	1.34 ± 0.12	4.70 ± 0.74	4.04 ± 0.44	6.15 ± 0.37	61.77**
13	ISOG (18:1)	-	-	-	-	0.45 ± 0.12	-	-	28.92**
14	Sum in 6	4.93 ± 1.22	7.21 ± 1.27	6.55 ± 0.38	6.76 ± 0.43	5.81 ± 0.34	4.63 ± 0.96	5.01 ± 0.38	2.42**
15	Sum in 7	-	-	-	-	-	-	2.40 ± 0.33	108.39 **
16	Sum in 9	1.01 ± 0.07	-	-	-	-	-	-	435.18 **
17	Unknown 11.541	-	-	-	-	3.63 ± 0.55	-	-	38.48 **
	Total	13.57	15.89	25.19	19.58	24.01	18.96	22.64	

Table 2: Percentage composition of unsaturated fatty acids from the 10 days old laboratory cyanobacterial culture (** - Significant at 1%; * - Significant at 5%; NS - Not significant).

Discussion

The fatty acid composition in all the species showed high levels of saturated fatty acids with the values ranging from 74.81 - 86.43%, whereas the levels of unsaturated fatty acids were generally low (13.57 - 25.19%) during the present study and similar results were recorded by [14]. Highest percentage of saturated fatty acid and lowest percentage of unsaturated fatty acids were recorded in *Synechocystis salina* and highest percentage of unsaturated fatty acids and lowest percentage saturated fatty acid recorded

in *Oscillatoria cartiana* (Figure 1). In cyanobacteria species such as *Dermocarpa*, *Xenococcus*, *Dermocarpella*, *Myxosarcina* and *Pleurocapsa* contained high proportion of saturated fatty acids [15]. In the present study, the contents of palmitic acid (C_{16:0}) was the highest in all the seven species and same results observed by [14,16]. The composition of fatty acid influenced by various environmental parameters. The fatty acid content in *Calothrix* sp., isolated from a rice field in Mexico, was influenced by nitrate content in the culture medium and its unsaturated fatty

acid content was more [17]. The fatty acid content also influenced by light, temperature and salinity [18-22] observed the fatty acid composition was influenced by the effluents. The marine cyanobacteria, *Phormidium* sp. and *Oscillatoria* sp. have shown high level of cis-palmitoleic acid [23,24]. In present observation, the palmitoleic acids are observed in a range between 1.22% and 8.79% of total fatty acids in *Oscillatoria salina* and *Synechocystis salina* respectively (Table 2). Thus in the present study the level of palmitoleic acid is low as compared to the previous report.

Figure 1

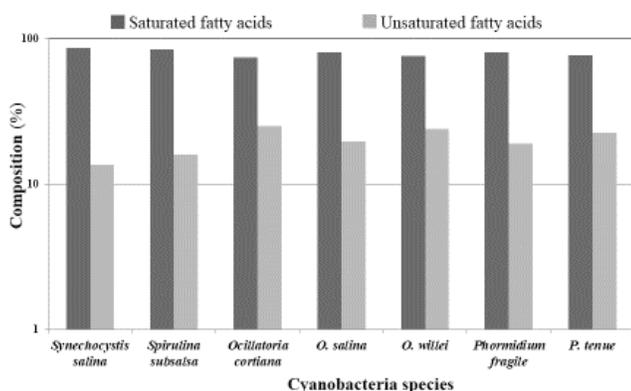


Figure 1: Shows the percentage composition of fatty acids from the 10 days old laboratory cyanobacterial culture.

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