ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 10: 275–289 (2024)
doi: 10.5281/zenodo.11063206
http://journal.asu.ru

RESEARCH ARTICLE

Fatty acid profiles in different phylogenetic and ecological groups of microalgae

Irina A. Maltseva[1], Alex V. Matsyura[2], Tatyana Yu. Gurova[1], Svetlana V. Cherkashina[1], Yevhen I. Maltsev[3]

1 *Melitopol State University, Melitopol*
2 *Altai State University, Barnaul*
3 *K.A. Timiryazev Institute of Plant Physiology RAS, Moscow*

Corresponding author: Alex V. Matsyura (amatsyura@gmail.com)

Academic editor: R. Yakovlev | Received 21 December 2023 | Accepted 19 January 2024 | Published 27 April 2024

http://zoobank.org/4308FDDB-D123-49F4-9AC2-A845708BD7E6

Citation: Maltseva IA, Matsyura AV, Gurova TYu, Cherkashina SV, Maltsev YeI (2024) Fatty acid profiles in different phylogenetic and ecological groups of microalgae. Acta Biologica Sibirica 10: 275–289. https://doi.org/10.5281/zenodo.11063206

Abstract

This study aimed to examine the fatty acid profiles of 10 newly discovered microalgae strains hailing from phylogenetic groups valued in biotechnology. The fatty acid profiles were characterized utilising principal component analysis, resulting in several notable findings. First, our analysis revealed that certain characteristics of these profiles align well with those previously identified in similar study groups. Most notably, the marine strain *Chlorella vulgaris* MI-Ch19-a was found to have the highest concentration of saturated fatty acids, measuring 60.48%. Furthermore, this strain also boasted the highest α-linolenic content among those analyzed, representing 22.14% of the total fatty acid spectrum. Each strain under study demonstrated significant amounts of 16:0 (with a range spanning 18.43% to 38.28%), 16:1n-7 (ranging from 17.05% to 32.55%), and 20:5n-3 (ranging from 4.96% to 20.13%). When considering the phylogenetic influence, which was particularly marked in the levels of total saturated fatty acids and polyunsaturated fatty acid content, it was the prominence of the n-3 polyunsaturated fatty acids that stood out amongst the phylogenetic groups. Lastly, the strains *Thalassiosira eccentrica* and *Cyclotella atomus* MI-B47 exhibited the highest volumes of eicosapentaenoic acid (20:5n-3).

Keywords

Freshwater algae, marine algae, multidimensional statistics, soil algae, biotechnology

Introduction

Microalgae, a versatile and abundant group of organisms, are found across various aquatic and terrestrial ecosystems. Their potential as a source of valuable bioactive compounds is increasingly appreciated, as highlighted in recent publications (Yaakob et al. 2021). These organisms are skilled at synthesising a vast array of fatty acids (FAs), which exhibit diverse properties and hold applications for biofuel production, food and feed additives, and the creation of pharmacological and cosmetic preparations (Sathasivam et al. 2019). Species of microalgae that accumulate large quantities of saturated and monounsaturated FAs are believed to hold potential for biofuel production. Meanwhile, species that amass long-chain polyunsaturated FAs, especially from the Omega-3 group, are considered valuable for procuring high-quality food products (Sajjadi et al. 2018; Maltsev et al. 2020). This has prompted interest in discovering new species and strains of microalgae with optimal FA compositions or the capacity for synthesising specific FAs in large volumes.

Several recent reviews have scrutinized the FA composition of microalgae (Lang et al. 2011; Galloway and Winder 2015; Cañavate 2018; Jónasdóttir 2019). Some suggest a degree of specificity in FA composition at the level of broad taxonomic groups (Taipale et al. 2013). Some put forth dependency on habitat for determining the predominance of certain FA groups (Galloway and Winder 2015; Cañavate 2018). Peltomaa et al. (2019) compared the profile and fatty acid content of strains from marine, brackish and freshwater habitats and concluded that although the fatty acid profiles are genetically ordered, the fatty acid content depended on the habitat by 39% and 59% in diatoms and dinoflagellates, respectively. There is also an ongoing debate about the influence of marine or freshwater origins of the strains on the FA composition, with differing conclusions. Addressing these discrepancies necessitates further research. A comprehensive understanding of FA compositions across varying taxonomic and ecological groups of microalgae is indispensable for several practical applications. It helps estimate the trophic value of diverse phytoplankton groups, monitor FA movement along food chains, and enhance quality of aquaculture feeds (Taipale et al. 2009; Sathasivam et al. 2019).

Progressing towards a unified understanding will also refine the bioprospecting strategy for identifying species and strains of biotechnologically valuable microalgae. Searches for strains with desired parameters can then be better targeted towards ecosystems. Thus, our work focuses on analyzing the FA profile of 10 new strains of freshwater, soil, and marine microalgae from relevant phylogenetic groups. Moreover, we aim to ascertain patterns in FA profiles and identify FAs that can serve as marker indicative of habitat or phylogenetic group among these new strains. Our study has implications for industrial biofuel production, food additives, and feed creation, including aquaculture. Additionally, our work showcases the importance of selecting an appropriate microalgae strain for producing specific FAs of interest.

Materials and methods

Algae cultures

Ten microalgae strains were selected for the experiment, which are stored in the CAMU collection and isolated from marine, freshwater and soil habitats (Table 1).

Microalgae cultivation

The marine microalgae were cultured in f/2 medium based on 10 psu of filtered and autoclaved Azov seawater (ASW), while the rest were cultured in BBM medium (Bischoff and Bold 1963) at 20 °C in a light-dark cycle (16:8 = light:dark) at a low light intensity of 70–100 μmol m^{-2} s^{-1}. Algae cultures were grown to an early stationary phase before being used in the experiment.

Table 1. Distribution of isolated microalgae species by taxonomic class

Taxa	Species	Strain	Geographic origin
Cyanophyceae	*Nostoc* sp.	MI-C84-a	The Azov Sea
Chlorophyceae	*Chlorococcum oleofaciens* Trainor et Bold	MZ-Ch4	Soil, Samara Forest
Trebouxiophyceae	*Chlorella vulgaris* Beijerinck	MI-Ch7-a	River Molochnaya
Trebouxiophyceae	*Chlorella vulgaris*	MI-Ch19-a	The Azov Sea
Chlorodendrophyceae	*Tetraselmis contracta* (N.Carter) Butcher	MI-Ch6-a	The Azov Sea
Bacillariophyceae	*Tabularia tabulata* (C.Agardh) Snoeijs	MI-B38	The Azov Sea
Bacillariophyceae	*Navicula cryptocephala* Kützing	MI-B42	The Azov Sea
Bacillariophyceae	*Cyclotella atomus* Hustedt	MI-B47	The Azov Sea
Bacillariophyceae	*Thalassiosira eccentrica* (Ehrenberg) Cleve	MI-B53	The Azov Sea
Cryptophyceae	*Hemiselmis* sp.	MI-C58	The Azov Sea

Fatty acid analysis

To determine the fatty acid composition, microalgae cells were centrifuged at 4000 rpm for 10 min and washed three times with distilled water. After washing, the algae biomass was immediately resuspended in 10 mL of hot isopropyl alcohol that contained 20 mg L^{-1} ionol at 70 °C for 20 min. The samples were stored at -20 °C until analysis. Lipid extracts were prepared by the Bligh and Dyer (1959) method with Palmer's recommendations (1971). The composition of FAs was determined in lipid extracts by gas-liquid chromatography using a Carlo Erba chromatograph

(Italy) with glass capillary columns (2.5 × 3 mm). Chromosorb W/DP with Silar 5CP phase (Serva, Germany) at an ion concentration of 10% at temperatures of 140 to 250 °C with steps of 2 °C min^{-1} (injector temperature 210 °C, detector temperature 240 °C) was used as a carrier.

Statistics

All measurements were made in three repetitions. Data are presented as mean values and standard errors. Statistical analysis was performed using XLSTAT 2018 (New York, USA). The data in graphs and tables are presented as mean values and standard deviations. Statistics were obtained in the Microsoft Excel program (ver. 1903) using one-factor analysis of variance (ANOVA). The reliability of the differences between the indices was calculated using the Tukey-Kramer post hoc test. The results with $p < 0.05$ were considered statistically significant. The relationships between parameters were analyzed with principal component analysis (PCA). Statistical calculations and graphing were performed using Statistica ver. 12.0 software.

Result

Twenty-three FAs containing between 12 and 22 carbon atoms in the chain were identified in ten microalgae strains. The composition of FA of the strains is presented in Table 2. Such FAs as 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6, and 18:3n-3 were observed in 80–90% of the studied samples and their number was significant and exceeded 1% in most cases. The group of rare FAs was formed by 12:0, 16:1n-5, 16:2n-4, 18:2n-9, 16:3n-3, 16:4n-3, and 22:6n-3, but its amount varied significantly in the strains studied. The strains also differed in the total amount of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Table 2).

Table 2. Fatty acid composition (as a percentage of total fatty acids) found in isolated microalgae kept in batch cultures. The letters indicate significant differences among species by fatty acid

	Species and Strain									
Fatty acids	*Nostoc* sp. MI-C84-a	*Chlorococcum oleofaciens* MZ-Ch4	*Chlorella vulgaris* MI-Ch7-a	*Chlorella vulgaris* MI-Ch19-a	*Tetraselmis contracta* MI-Ch6-a	*Tabularia tabulata* MI-B38	*Navicula cryptocephala* MI-B42	*Cyclotella atomus* MI-B47	*Thalassiosira eccentrica* MI-B53	*Hemiselmis* sp. MI-C58
12:0	n.d.*	0.13± 0.01	n.d.	3.1±0.52	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14:0	1.14± 0.12	0.15± 0.01	n.d.	0.27± 0.01	0.5±0.03	4.58± 0.65	2.72± 0.42	1.17± 0.88	15.7±1.32	1.57± 0.85

Fatty acids	Species and Strain									
	Nostoc sp. MI-C84-a	*Chlorococcum oleofaciens* MZ-Ch4	*Chlorella vulgaris* MI-Ch7-a	*Chlorella vulgaris* MI-Ch19-a	*Tetraselmis contracta* MI-Ch6-a	*Tabularia tabulata* MI-B38	*Navicula cryptocephala* MI-B42	*Cyclotella atomus* MI-B47	*Thalassiosira eccentrica* MI-B53	*Hemiselmis* sp. MI-C58
16:0	22.63± 3.52	18.43± 2.15	17.53± 2.54	24.08± 4.11	21.32± 3.15	27.11± 4.14	38.28± 3.52	21.15± 2.19	18.43± 2.34	18.24± 2.91
18:0	62.28± 5.66	35.97± 3.72	16.04± 1.13	33.03± 2.15	1.25± 0.42	16.18± 1.17	6.83± 0.75	n.d.	2.32± 0.55	n.d.
16:1n-9	n.d.	n.d.	n.d.	1.16± 0.18	3.15± 0.73	7.85± 0.77	n.d.	n.d.	n.d.	5.83± 0.58
16:1n-7	4.13± 0.52	1.57± 0.19	0.95± 0.02	0.61± 0.01	2.93± 0.16	17.05± 1.15	32.55± 2.11	29.85± 3.14	21.18± 2.17	n.d.
16:1n-5	n.d.	0.71± 0.01	0.54± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18:1n-9	1.21± 0.22	2.86± 0.73	12.33± 1.22	12.88± 1.67	7.85± 0.92	3.71± 0.73	2.19± 0.82	n.d.	1.34± 0.32	1.54± 0.41
18:1n-7	n.d.	12.75± 1.52	n.d.	n.d.	3.53± 0.62	n.d.	1.29± 0.55	n.d.	n.d.	n.d.
20:1n-9	n.d.	n.d.	n.d.	n.d.	2.15± 0.62	n.d.	n.d.	n.d.	n.d.	8.47± 0.78
16:2n-6	n.d.	3.04± 0.42	7.5± 0.64	n.d.	n.d.	0.35± 0.01	1.33± 0.11	5.84± 0.43	n.d.	n.d.
16:2n-4	n.d.	n.d.	n.d.	n.d.	n.d.	3.11±0.32	n.d.	n.d.	2.23± 0.22	n.d.
18:2n-9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.13± 0.19	n.d.	n.d.	n.d.
18:2n-6 LA	4.35± 0.82	5.98± 0.55	20.41± 3.15	7.3± 0.92	4.42± 0.67	1.22±0.14	2.41± 0.11	n.d.	1.2± 0.08	4.26± 0.14
16:3n-4	n.d.	n.d.	n.d.	n.d.	n.d.	4.52±0.52	0.36± 0.02	10.55± 0.74	10.35 ±1.02	n.d.
16:3n-3	n.d.	3.81± 0.42	4.51± 0.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18:3n-6 GLA	n.d.	3.22± 0.56	n.d.	3.29± 0.72	0.35± 0.03	0.78±0.04	0.48± 0.01	n.d.	n.d.	n.d.
18:3n-3 ALA	1.21± 0.22	8.63± 0.54	18.07± 1.13	13.15± 1.22	22.14± 2.15	n.d.	0.85± 0.02	n.d.	3.54± 0.45	12.48± 1.37
16:4n-3	n.d.	n.d.	n.d.	n.d.	14.82± 1.18	n.d.	n.d.	1.15± 0.35	n.d.	n.d.
18:4n-3 SDA	n.d.	n.d.	n.d.	n.d.	6.33± 0.73	0.85± 0.09	1.17± 0.13	8.84± 0.78	4.15± 0.75	28.1± 1.84
20:4n-6 ARA	n.d.	n.d.	n.d.	n.d.	0.56± 0.03	6.97± 0.75	n.d.	n.d.	0.42± 0.01	n.d.
20:5n-3 EPA	n.d.	n.d.	n.d.	n.d.	7.1±0.42	4.96± 0.15	5.31± 0.84	20.13± 1.65	15.36± 1.32	15.52± 1.89
22:6n-3 DHA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.04± 0.02	n.d.	2.28± 0.15	n.d.

Fatty acids	Species and Strain									
	Nostoc sp. MI-C84-a	*Chlorococcum oleofaciens* MZ-Ch4	*Chlorella vulgaris* MI-Ch7-a	*Chlorella vulgaris* MI-Ch19-a	*Tetraselmis contracta* MI-Ch6-a	*Tabularia tabulata* MI-B38	*Navicula cryptocephala* MI-B42	*Cyclotella atomus* MI-B47	*Thalassiosira eccentrica* MI-B53	*Hemiselmis* sp. MI-C58
Other	3.05± 0.72	1.28± 0.05	2.12± 0.22	1.13± 0.15	1.6±0.18	0.76± 0.02	2.06± 0.12	2.32± 0.54	1.15± 0.35	3.95± 0.83
∑SFA	86.05± 7.32	54.68± 4.74	33.57± 3.16	60.48± 5.51	23.07± 3.72	47.87± 4.13	47.83± 3.56	22.32± 2.26	36.45± 3.72	19.81± 2.23
∑MUFA	5.34± 0.86	17.89± 2.86	13.82± 1.72	14.65± 1.46	19.61± 2.14	28.61± 1.86	36.03± 3.72	29.85± 3.14	22.52± 2.26	15.84± 1.22
∑PUFA	5.56± 0.76	24.68± 3.47	50.49± 4.35	23.74± 1.65	55.72± 3.78	22.76± 1.26	14.08± 0.96	46.51± 2.48	39.53± 2.34	60.4± 3.87
∑n-3 PUFA	1.21± 0.22	12.44± 0.76	22.58± 2.16	13.15± 1.22	50.39± 3.71	5.81± 0.23	8.37± 0.78	30.12± 2.26	25.33± 1.96	56.14± 3.75
∑n-6 PUFA	4.35± 0.82	12.24± 0.84	27.91± 2.64	10.59± 1.56	5.33± 0.65	12.43± 0.82	4.22± 0.11	5.84± 0.43	1.62± 0.08	4.26± 0.14
n-3:n-6	0.28	1.02	0.81	1.24	9.45	0.47	1.98	5.16	15.64	13.18

Note: *n.d. not detected.

Cyanophyceae

The marine strain *Nostoc* sp. MI-C84-a is characterized by a high SFA content in the FA spectrum. The leading position is occupied by 18:0, accounting for 62.28% of all FAs. Of the MUFAs and PUFAs, 16:1n-7 and 18:2n-6 are predominant. PUFA n-3 and PUFA n-6 are restricted to linoleic (LA) and α-linolenic (ALA) acids only (Table 2).

Green microalgae

Of the green microalgae, strains from different classes and different habitats were studied. The mean content of green microalgae SFA was 42.95%, MUFA was 16.49%, and PUFA was 38.66%. The highest SFA content was found for the marine strain *Chlorella vulgaris* MI-Ch19-a at 60.48%. In the freshwater strain, the amount of SFA was almost two times less (Table 2). Another characteristic of the freshwater strain was the higher content of PUFA, and among them, 18:2n-6 and 18:3n-3.

Chlorococcum oleofaciens MZ-Ch4 was also characterized by a high amount of SFA. The basis of the FA spectrum of *Chlorococcum oleofaciens* MZ-Ch4 comprised 18:0, 16:0, 18:1n-7 and 18:3n-3. The total content of n-3 PUFA and n-6 PUFA was close to 12.24_12.44%, with ALA predominating, accounting for 8.63% of the total spectrum of FA.

The spectrum of FA of *Tetraselmis contracta* MI-Ch6-a differed from other green microalgae by the low content of SFA and MUFA and high amount of PUFA (Table 2). At the level of individual FAs, 16:0 of SFA, 18:1n-9 of MUFA, and 18:3n-3, 16:4n-3 of PUFA were predominant. The total PUFA content for n-3 was more than five times higher than that for n-6 PUFA. The amount of ALA was 22.14% of the total spectrum of FAs and was the highest among the strains studied in this work.

Bacillariophyceae

The four diatom strains studied had an average SFA content of 38.62%, a MUFA content of 29.25% and a PUFA content of 30.72%. All strains were characterized by a high content of 16:0 (from18.43% to 38.28%), 16:1n-7 (from 17.05% to 32.55%), and 20:5n-3 (from 4.96% to 20.13%). *Navicula cryptocephala* MI-B42 had the lowest PUFA (14.08%) and *Cyclotella atomus* MI-B47 was the highest (46.51%). Furthermore, *Cyclotella atomus* MI-B47 had the highest n-3 PUFA content of 30.12% (eicosapentaenoic acid (EPA) 20.13%, stearidonic acid (SDA) 8.84%). *Thalassiosira eccentrica* MI-B53 had slightly lower (25.33%) PUFA content, but was dominated by EPA with 15.36% and SDA with 4.15%. *Tabularia tabulata* MI-B38 was distinctive for its high n-6 PUFA content (12.43%). Among the n-6 PUFAs, ARA was predominant (6.97%).

Cryptophyceae

Hemiselmis sp. MI-C58 differed from all strains studied in the highest PUFA content of 60.4%, among which n-3 PUFAs were predominant. SDA (28.1%), EPA (15.52%) and ALA (12.48%) were the leading in terms of number. The n-6 PUFAs were only represented by LA with 4.26%.

Phylogeny and habitat explaining proportional fatty acid profiles

The principal component analysis for the entire group of variables showed that the first two components accounted for 78.18% of the total changes observed in the PCA (Fig. 1). In this analysis, the greatest contribution with a negative correlation coefficient in PCA 1 is the belonging of microalgae strains to a particular phylogenetic group, with a positive one in the number of SFAs and negative ones in the number of PUFAs in general, with n-3 PUFAs and the ratio n-3:n-6. PCA 2 shows the main patterns of variation in FA profile associated with habitat conditions. The greatest dependence on habitat conditions is shown by the amount of n-6 PUFA, which is also shown by the moderate strength of the relationship with the total content of PUFA. PCA 3 describes only 14.96% of the total variance, but because it has an eigenvalue greater than 1, it was selected for analysis. This component reflects the MUFA content and in this study has little relationship with both the strain's habitat conditions and its phylogenetic group membership.

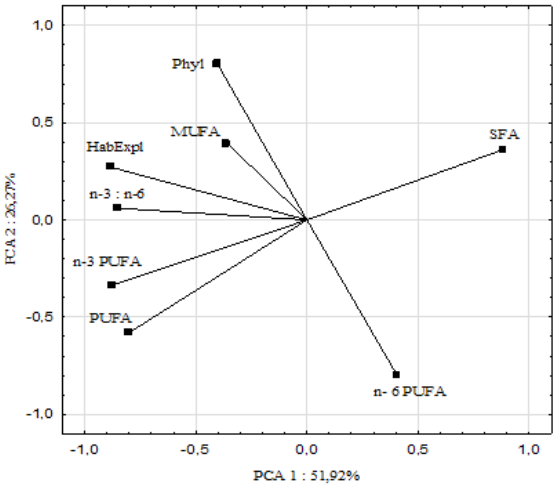

Figure 1. PCA plot of microalgae strains based on their fatty acid profiles: SFA, MUFA, PUFA, n-3 PUFA, n-6 PUFA – total amount of SFA, MUFA, PUFA, n-3 PUFA, n-6 PUFA, respectively; n-3:n-6 is the ratio of n-3 PUFA to n-6 PUFA; Phyl – the class level of the phylogenetic group membership of the strain; HabExpl – habitat conditions of the strain.

Discussion

Microalgae have the ability to synthesize various fatty acids (FAs) found in cells in the free state or as part of various lipids, including triacylglycerols (TAG), phospholipids, and glycolipids (Li-Beissona et al. 2019). The specific properties of these compounds are largely determined by the FAs that comprise them, and the properties of FAs depend on the length of the hydrocarbon chain, the presence, location, and number of double bonds between carbon atoms, the presence of specific functional groups, and branching of the hydrocarbon chain. Different groups of lipids are not identical in terms of FA composition. For example, previous studies have found that TAGs predominantly contain saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), while polar lipids contain polyunsaturated fatty acids (PUFA) (Xin et al. 2019; Harwood 2019). Moreover, recent reviews have reported that microalgae also exhibit a variation in FA profile depending on their habitat, including characteristics such as saturated, unsaturated, and long-chain FA content (Sharathchandra and Rajashekhar 2011; Galloway and Winder 2015; Cañavate 2018).

Our studies also suggest that there are consistent features in the FA composition of microalgae, as established by previous studies for certain phylogenetic groups. However, variations in FA composition related to the peculiarities of the strain's habitat conditions are also observed. The principal component analysis (PCA) results showed that phylogenetic dependence is most pronounced at the level of the total content of SFA and PUFA, and among the latter, at the amount of n-3 PUFA.

The habitat conditions of the microalgae strain had the greatest influence on the amount of n-6 PUFA. Changes in FA composition with variations in temperature, availability of nutritional elements in microalgae culture, and salinity of the medium have been well described (Cordeiro et al. 2017; Maltsev et al., 2018; Aboim et al., 2019). For instance, when marine strains were grown under different salinity conditions (10 to 35 psu), *Desmodesmus* sp. nl3 showed an increase in MUFA from 9.47% to 29.85% and a decrease in PUFA from 71.53% to 48.26% (Luu et al. 2020). This increase in unsaturated FAs has also been observed in *Ulva intestinalis* Linnaeus (Nesterov et al. 2013), indicating that n-3 PUFA and n-6 PUFA are involved in the adaptation of the alga to changes in habitat conditions, including water salinity, temperature, oxygen saturation, and acidity of the medium.

As a distinct phylogenetic group, cyanobacteria are characterized by the predominance of 16:0, 16:1 and FAs with 18 carbon atoms (Lang et al. 2011). Depending on the number of certain FAs in the profile, 4 to 5 groups have been proposed among cyanobacteria (Kenyon et al. 1972; Cohenet et al. 1995). The strain studied *Nostoc* sp. MI-C84-a belongs to the first group, which is characterized by a high content of saturated and monounsaturated FAs: 16:1n-7, 18:1n-9. The peculiarity of the profile of *Nostoc* sp. MI-C84-a has the highest content of 18:0 compared to other species of this genus, which is dominated by 16:0 among SFA (Gugger et al. 2002; Temina et al. 2007; Tiwari and Sharma 2021). The content of cyanobacterial specific 16:1n-7, 18:1n-9 in the studied strain is lower than what is known, for example, for marine *Nostoc calcicola* Brébisson ex Bornet et Flahault and *Nostoc commune* Vaucher ex Bornet et Flahault with values of 7.05%, 28.29% and 13.5%, 4.1%, respectively. At the same time, many freshwater *Nostoc* strains do not show FAs such as 16:1n-7 and 18:1n-9 in their FA profile (Temina et al. 2007). Higher content of 18:3n-6 has been identified as another freshwater Cyanophyceae compared to marine ones (Cañavate 2018). The new strain is superior to the known *Nostoc* in total SFA content. The predominance of FAs with 16 and 18 carbon atoms in the profile is considered a common feature of a large phylogenetic group of green microalgae. At the class level, the predominance of 16:2 for Trebouxiophyceae was registered, while 16:3 and 16:4 were observed in Chlorophyceae and Chlorodendrophyceae. These classes are also characterized by 18:1n-9, 18:2n-6, 18:n-3, and 22:6n-3 (docosahexaenoic acid (DHA)) (Jónasdóttir 2019). In general, the profile of the green microalgae studied in this work is characterized by the predominance of FA with 16 and 18 carbon atoms, except *Tetraselmis contracta* MI-Ch6-a, which also presents FA with 20 and 22 carbon atoms.

The profile of *Chlorococcum oleofaciens* MZ-Ch4 is characterized by a high amount of SFA (54.68%), which distinguishes it from another strain of *Chlorococcum oleofaciens* SAG 213-11 containing 26.28% SFA (Del Río et al., 2017), as well as from *Chlorococcum amblystomatis* (F.D.Lambert ex N.Wille) N.Correia, J.Varela et Leonel Pereira containing 38.9% SFA (Cordeiro et al. 2017). The closest SFA content to our data was observed in *Chlorococcum infusionum* (Schrank) Meneghini SAG 10.86 with 45.6% (Lang et al. 2011). A specific feature of the new strain of *Chlorococ-*

cum oleofaciens MZ-Ch4 was the high content of ALA (18:3n-3) – 8.68%. Previously, the ALA content of 5.45% was reported for *Chlorococcum oleofaciens* SAG 213-11 and 3.2% for *Chlorococcum amblystomatis* (Del Río et al. 2017; Cordeiro et al. 2017).

In this study, the marine strain *Chlorella vulgaris* MI-Ch19-a differed significantly from freshwater MI-Ch7-a in SFA and PUFA content. High content of palmitic and stearic acids (up to 57.8% or higher) in marine *Chlorella* sp. and *Chlorella vulgaris* was also observed in previous reports (Amaral et al. 2015; Kurnia et al. 2017). However, other studies reported that marine *Chlorella vulgaris* had lower amounts of SFA than in our study, varying from 21.35% to 34.9% (Pratoomyot et al. 2005; Petkov and Garcia 2007). The FA content of the n-3 PUFA was higher in the studied freshwater strain *Chlorella vulgaris* MI-Ch7-a than in the marine strain and reached 22.58%. As reported by Freitas (2017) in *Chlorella* Beyerinck [Beijerinck] cells, n-3 PUFAs and n-6 PUFAs can account for up to 35–40% of total lipid content. This accounts for the high value of *Chlorella* species as sources of such FAs and primarily linoleic acid and α-linolenic acid. The content of these FAs in some strains of *Chlorella vulgaris* can reach 24% and 27% (Petkov and Garcia 2007). For the freshwater strain in our studies, the content of LA and ALA was also high, 20.41% and 18.04%, respectively.

In terms of FA composition, *Tetraselmis contracta* MI-Ch6-a is comparable to previous reports but is characterized by a higher PUFA content than *Tetraselmis suecica* (Kylin) Butcher, Tetraselmis sp. in which this index was 17.2% and 33.51%, respectively (Pratoomyot et al. 2005; Jiménez-Valera et al. 2016). In our studies, no predominance of palmitic acid (16:0) was observed, which accumulated at 54.49% in the *Tetraselmis* sp. strain from Indonesia (Widianingsih et al. 2013) and 30.3% in the strain of *Tetraselmis suecica* from Mexico (Jiménez-Valera et al. 2016). The composition of PUFA was dominated by ALA and lacked DHA, which was also observed for *Tetraselmis* sp. (Pratoomyot et al. 2005). In general, this strain of *Tetraselmis contracta* MI-Ch6-a contained the highest amount of PUFAs among the green microalgae studied, especially n-3 PUFAs, which is important from the perspective of microalgae utilization technologies to correct the disturbed omega-3: omega-6 balance in diets through its inclusion in food and feed additives (Simopoulos 2016).

Diatoms are believed to be dominated by FAs with 16 carbon atoms and especially 16:1n-7 is formed in large quantities (Jónasdóttir 2019). In Bacillariophyceae in this study, 16:0, 16:1n-7, and 20:5n-3 were the main FAs. The content of 16:1n-7 ranged from 17.05% to 32.55%. SFAs represented about all FAs in *Tabularia tabulata* MI-B38 and *Navicula cryptocephala* MI-B42, while PUFAs dominated in *Thalassiosira eccentrica* MI-B53 and *Cyclotella atomus* MI-B47.

Thalassiosira eccentrica MI-B53 and *Cyclotella atomus* MI-B47 had the highest amount of EPA (20:5n-3), which was higher than previously reported for *Thalassiosira* sp. (Pratoomyot et al. 2005), *Stephanocyclus cryptica* (Reimann, Levin et Guillard) Kulikovskiy, Genkal et Kociolek (Pahl et al. 2010). The amount of DHA

(22:6n-3) in *Thalassiosira eccentrica* MI-B53 was higher than previously reported for different strains of *Thalassiosira* sp. (Pratoomyot et al. 2005; Widianingsih et al. 2013). A feature of the FA profile of *Tabularia tabulata* MI-B38 was the high ARA content, higher than that of the strains studied in this study, as well as *Stephanocyclus cryptica* (Pahl et al. 2010), *Thalassiosira* sp., *Nitzschia* cf. *ovalis* (Pratoomyot et al. 2005).

The marine strain *Navicula cryptocephala* MI-B42 contained more palmitic acid (16:0) in our studies than the freshwater *Navicula cryptocephala* 33.9% (Sanjay et al. 2013). The FA amount of 16:1n-7 in the studied strain was higher than the freshwater strain containing 10.92% (Sanjay et al. 2013) and was close to marine strains of *Navicula* sp. (Jiménez-Valera et al. 2016). Our results are in agreement with a previous finding that freshwater Bacillariophyceae accumulate more 16:1n-7 compared to marine Bacillariophyceae (Cañavate 2018). Diatom strains and *Hemiselmis* sp. MI-C58 showed the highest number of FA with a chain with more than 18 carbon atoms in the profile, which is consistent with the pattern described previously for Bacillariophyceae and Cryptophyceae (Taipale et al. 2013; Jónasdóttir 2019). Cryptophyceae species are also known to have a high PUFA content (Peltomaa et al. 2017). The strain *Hemiselmis* sp. MI-C58 was characterized by a maximum PUFA content of 60.4% among those studied, among which n-6 PUFAs formed the basis. SDA (28.1%), EPA (15.52%), and ALA (12.48%) occupied the leading positions in terms of quantity. The n-6 PUFAs were only represented by LA with 4.26%. These characteristics are important in determining the directions of practical use in the future of the strains studied.

Overall, our study provides valuable insights into the FA composition of various microalgae strains, highlighting their potential for biotechnological applications, such as biofuel production, aquaculture feed, and pharmaceuticals. Further research into the lipid metabolism and genetic factors influencing FA composition in microalgae could lead to the development of tailored cultivation strategies for optimizing desired FA profiles.

Conclusion

The composition of the main groups and individual Fatty Acids (FAs) is reliant not only on their specific phylogenetic (taxonomic) group affiliation but also on their habitat or cultivation conditions. This suggests that variations in FA composition could represent an adaptive mechanism that enables algae to survive under different circumstances. Furthermore, n-6 Polyunsaturated Fatty Acids (PUFAs) are likely to have a significant function in this adaptation process.

Future research should aim to further clarify the relationship between the FA profile and phylogeny and consider the impact of environmental aspects. This will enable the creation of robust algorithms for evaluating the trophic value of differ-

ent phytoplankton groups. Additionally, it will guide bioprospecting strategies for finding biotechnologically valuable species and strains of microalgae. Such studies hold immense potential for expanding our understanding of diverse phytoplankton groups and fostering biotechnological advancements through algae.

Acknowledgements

This publication is based on research carried out with financial support from the Russian Science Foundation (project 23-74-10081). Isolation of microalgae and cyanobacteria strains was maintained within the theme FRRS-2023-0035. The manuscript design was obtained within the State Assignment of the Ministry of Science and Higher Education of the Russian Federation (theme 122042700045-3).

References

Aboim JB, Oliveira DTd, Mescouto VAd, dos Reis AS, da Rocha Filho GN, Santos AV, Xavier LP, Santos AS, Gonçalves EC, do Nascimento LAS (2019) Optimization of light intensity and NaNO3 concentration in amazon cyanobacteria cultivation to produce biodiesel. Molecules 24(12): 2326. https://doi.org/10.3390/molecules24122326

Amaral MS, Loures CC, Da Rós PCM, Machado SA, Reis CER, de Castro HF, Silva MB (2015) Evaluation of the cultivation conditions of marine microalgae *Chlorella* sp. to be used as feedstock in in ultrasound-assisted ethanolysis. Biofuel Research Journal 7: 288–294. https://doi.org/10.18331/BRJ2015.2.3.7

Bischoff HW, Bold HC (1963) Phycological studies IV. Some soil algae from Enchanted Rock and related algal species. University of Texas Publication 6318: 95.

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911–917. https://doi.org/10.1139/o59-099

Cañavate JP (2018) Advancing assessment of marine phytoplankton community structure and nutritional value from fatty acid profiles of cultured microalgae. Reviews in Aquaculture 11(3): 527–549. https://doi.org/10.1111/raq.12244

Cohen Z, Norman HA, Heimer YM (1995) Microalgae as a source of ω 3 fatty acids. In: Simopoulos AP (Ed.) Plants in Human Nutrition. Karger, Basel 77: 1–31. https://doi.org/10.1159/000424462

Cordeiro RS, Vaz ICD, Magalhães SMS, Barbosa FAR (2017) Effects of nutritional conditions on lipid production by cyanobacteria. Anais da Academia Brasileira de Ciencias 89(3 Suppl): 2021–2031. https://doi.org/10.1590/0001-3765201720150707

Del Río E, García-Gómez E, Moreno J, Guerrero MG, García-González M (2017) Microalgae for oil. Assessment of fatty acid productivity in continuous culture by two high-yield strains, *Chlorococcum oleofaciens* and *Pseudokirchneriella subcapitata* Algal Research 23: 37–42. http://dx.doi.org/10.1016/j.algal.2017.01.003

Domingues MR (2021) Microalgae as sustainable bio-factories of healthy lipids: evaluating fatty acid content and antioxidant activity. Marine Drugs 19: 357. https://doi.org/10.3390/md19070357

Freitas HR (2017) *Chlorella vulgaris* as a Source of Essential Fatty Acids and Micronutrients: A Brief Commentary. The Open Plant Science Journal 10: 93–99. https://doi.org/10.2174/1874294701710010092

Galloway AW, Winder M (2015) Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLoS One 10:e0130053. https://doi.org/10.1371/journal.pone.0130053

Gugger M, Lyra C, Suominen I, Tsitko I, Humbert JF, Salkinoja-Salonen MS, Sivonen K (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (Cyanobacteria). International Journal of Systematic and Evolutionary Microbiology 52: 1007–1015. https://doi.org/10.1099/00207713-52-3-1007

Harwood JL (2019) Algae: critical sources of very long-chain polyunsaturated fatty acids. Biomolecules 9: 708. https://doi.org/10.3390/biom9110708

Jiménez-Valera S, Sánchez-Saavedra MP (2016) Growth and fatty acid profiles of microalgae species isolated from the Baja California Peninsula, México. Latin American Journal of Aquatic Research 44(4): 689–702. https://doi.org/10.3856/vol44-issue4-fulltext-4

Jónasdóttir SH (2019) Fatty acid profiles and production in marine phytoplankton. Marine Drugs 17: 151. https://doi.org/10.3390/md17030151

Kenyon CN, Rippka R, Stanier RY (1972) Fatty acid composition and physiological properties of some filamentous blue-green algae. Archiv Mikrobiol 83: 216–236. https://doi.org/10.1007/BF00645123

Kurnia D, Yuliantini A, Cendana IS, Nurachman Z (2019) Fatty acid analysis of marine microalgae *Chlorella vulgaris* in modified medium used GC-FID. Journal of Physics: Conf. Series 1338 (2019): 012007. https://doi.org/10.1088/1742-6596/1338/1/012007

Lang I, Hodac L, Friedl T, Feussner I (2011) Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biol 11: 124. https://doi.org/10.1186/1471-2229-11-124

Li-Beissona Y, Thelen JJ, Fedosejevs E, Harwood JL (2019) The lipid biochemistry of eukaryotic algae. Progress in Lipid Research 74: 31–68. https://doi.org/10.1016/j.plipres.2019.01.003

Luu TN, Alsafra Z, Corato A, Corsaro D, Le HA, Eppe G, Remacle C (2020) Isolation and characterization of two microalgal isolates from Vietnam with potential for food, feed, and biodiesel production. Energies 13: 898. https://doi.org/10.3390/en13040898

Maltsev YI, Gusev E, Maltseva IA, Kulikovskiy M, Namsaraev Z, Petrushkina M, Filimonova A, Sorokin B, Golubeva A, Butaeva G, Khrushchev A, Zotko N, Kuzmin D (2018) Description of a new species of soil algae, *Parietochloris grandis* sp. nov., and study of its fatty acid profiles under different culturing conditions. Algal Research 33: 358–368. https://doi.org/10.1016/j.algal.2018.06.008

Maltsev YI, Maltseva IA, Maltseva SY, Kulikovskiy MS (2020) Biotechnological potential of a new strain of *Bracteacoccus bullatus* (Sphaeropleales, Chlorophyta) as a promising

producer of omega-6 polyunsaturated fatty acids. Russian Journal of Plant Physiology 67: 185–193. https://doi.org/10.1134/S1021443720010124

Nesterov VN, Rozentsvet OA, Bogdanova ES (2013) The influence of abiotic factors on the composition of fatty acids in *Ulva intestinalis*. Siberian Ecological Journal 4: 585–592. https://doi.org/10.1134/S1995425513040070 [In Russian]

Pahl SL, Lewis DM, Chen F, King KD (2010) Growth dynamics and the proximate biochemical composition and fatty acid profile of the heterotrophically grown diatom *Cyclotella cryptica*. Journal of Applied Phycology 22: 165–171. https://doi.org/10.1007/s10811-009-9436-7

Palmer FBS-C (1971) The extraction of acidic phosphollpids in organic solvent mixtures containing water. Biochimica et Biophysica Acta 231(1): 134–144. https://doi.org/10.1016/0005-2760(71)90261-x

Peltomaa ET, Hällfors H, Taipale SJ (2019) Comparison of diatoms and dinoflagellates from different habitats as sources of PUFAs. Marine Drugs 17: 233. https://doi.org/10.3390/md17040233

Petkov G, Garcia G (2007) Which are fatty acids of the green alga Chlorella? Biochemical Systematics and Evolution 35: 281–285. https://doi.org/10.1016/j.bse.2006.10.017

Pratoomyot J, Srivilas P, Noiraksar T (2005) Fatty acids composition of 10 microalgal species. Songklanakarin Journal of Science and Technology 27(6): 1179–1187.

Sajjadi B, Chen WY, Raman AAA, Ibrahim S (2018) Microalgae lipid and biomass for biofuel production: a comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. Renewable and Sustainable Energy Reviews 97: 200–232. https://doi.org/10.1016/J.RSER.2018.07.050

Sanjay KR, Prasad MNN, Anupama S, Yashaswi BR, Deepak B (2013) Isolation of diatom *Navicula cryptocephala* and characterization of oil extracted for biodiesel production. African Journal of Environmental Science and Technology 7(1): 41–48. https://doi.org/10.5897/AJEST12.066

Sathasivam R, Radhakrishnan R, Hashem A, Abd Allah EF (2019) Microalgae metabolites: a rich source for food and medicine. Saudi Journal of Biological Sciences 26: 709–722. https://doi.org/10.1016/j.sjbs.2017.11.003

Sharathchandra K, Rajashekhar M (2011) Total lipid and fatty acid composition in some freshwater cyanobacteria. Journal of Algal Biomass Utilization 2: 83–97.

Simopoulos AP (2016) An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 8(3): 128. https://doi.org/10.3390/nu8030128

Taipale SJ, Kankaala P, Hämäläinen H, Jones RI (2009) Seasonal shifts in the diet of lake zooplankton revealed by phospholipid fatty acid analysis. Freshwater Biology 54: 90–104. https://doi.org/10.1111/j.1365-2427.2008.02094.x

Taipale SJ, Strandberg U, Peltomaa E, Galloway AWE, Ojala A, Brett MT (2013) Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquatic Microbial Ecology 71: 165–178. https://doi.org/10.3354/ame01671

Temina M, Rezankova H, Rezanka T, Dembitsky VM (2007) Diversity of the fatty acids of the *Nostoc* species and their statistical analysis. Microbiological Research 162(4): 308–321. https://doi.org/10.1016/j.micres.2006.01.010

Tiwari P, Sharma PK (2021). Effect of temperature on fatty acid profile of *Nostoc spongiaeforme* (freshwater) and marine water *Nostoc calcicola* (marine water): A comparative study. Journal of Stress Physiology & Biochemistry 17(4): 119–125.

Widianingsih, Hartati R, Endrawati H, Mamuaja J (2013) Fatty acid composition of marine microalgae in Indonesia. Journal of Tropical Biology and Conservation 10: 75–82.

Xin Y, Shen C, She Y, Chen H, Wang C, Wei L, Yoon K, Han D, Hu Q, Xu J (2019) Biosynthesis of triacylglycerol molecules with a tailored PUFA profile in industrial microalgae. Molecular Plant 12: 474–488. https://doi.org/10.1016/j.molp.2018.12.007

Yaakob MA, Mohamed RMSR, Al-Gheethi A, Aswathnarayana Gokare R, Ambati RR (2021) Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: an overview. Cells 10(2): 393. https://doi.org/10.3390/cells10020393