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EXPERIMENTAL ARTICLES

Secretion of Free Fatty Acids by Prokaryotic and Eukaryotic Algae at Optimal, Supraoptimal, and Suboptimal Growth Temperatures

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Abstract—The paper describes the composition of extracellular free fatty acids (FFAs) and intracellular fatty acids (FAs) in the enrichment cultures of the prokaryotic alga *Spirulina platensis* and the eukaryotic alga *Chlorella vulgaris* grown at optimal, supraoptimal, and suboptimal growth temperatures. With increasing growth temperature, the degree of unsaturation of the intracellular FAs of both algae decreased, while that of the extracellular FFAs of *S. platensis* increased. The composition of the extracellular FFAs of *C. vulgaris* practically did not depend on the growth temperature.

Key words: free fatty acids, secretion, microalgae.

Free fatty acids (FFAs) are important physiological and ecological markers. Data on the fatty acid (FA) composition of bacterial, algal, and plant cells are efficiently used in taxonomy [1, 2] and for the identification of organic matter sources in aquatic and soil ecosystems [3, 4]. The composition of extracellular FFAs in the surface film of bodies of water is a valuable ecological marker of seasonal plankton succession and the kinetics of water clarification [5, 6]. In order to use the composition of extracellular FFAs for hydroecological monitoring, the sources and the mechanisms of their secretion must be understood. According to some data in the literature, the primary source of FFAs in natural bodies of water is their secretion by phytoplankton [5–7].

The secretion of organic substances into the surrounding medium is a normal physiological process in algae and cyanobacteria [8]. The ecological role of this process is known only for specific groups of substances, such as enzymes and polysaccharides [8, 9]. At the same time, the role of the secretion of lipids, including FFAs, remains almost unknown. FFAs are an important component of the organic matter of natural bodies of water [10], and the composition of FFAs in a body of water reflects the state of its phytoplankton [11]. In view of this, the study of the mechanisms of FFA secretion is of great biochemical and ecological significance.

Water temperature influences lipid metabolism in cells and considerably affects the composition and the physiological state of phytoplankton [12]. Data on the effect of temperature on the secretion and composition of extracellular FFAs is scarce [13]. Relevant studies along this line of research obviously must include both the eukaryotic and prokaryotic types of phytoplankton, since their lipid metabolism pathways significantly differ [12].

The aim of the present work was to study the effect of growth temperature on the composition of intracellular FAs and on the secretion of extracellular FFAs in the eukaryotic green alga *Chlorella vulgaris* and the prokaryotic blue–green alga *Spirulina platensis.*

MATERIALS AND METHODS

Cultures and cultivation conditions. The blue–green alga (cyanobacterium) *Spirulina platensis* (Nordst) Geitl and the green alga *Chlorella vulgaris* Beijer were obtained from the collection of microalgae at the Institute of Biophysics. The algae were grown in a batch mode as described elsewhere [14, 15]. Cells for inoculation were grown for the total 10 days with two subsequent transfers to fresh media. The initial concentration of the inoculated exponential-phase algal cells was 50–80 mg dry wt/l. Cultivation flasks with a volume of 1 l were placed in a water bath with transparent walls and incubated at 6000-lx illumination with continuous air bubbling.

The cyanobacterium *S. platensis* was cultivated at three growth temperatures—suboptimal (30°C), optimal (35°C) [14], and supraoptimal (40°C). The corresponding growth temperatures for the green alga *C. vulgaris* were 20, 25, and 30°C. The temperature was controlled within an accuracy of ±0.2°C.

The experiments were performed as follows: Each alga was grown in triplicate flasks for 4–5 days at the optimum growth temperature. Then the culture from each flask was dispensed, in equal 500-ml portions, into two new flasks and diluted twofold with fresh growth

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medium. Subsequently, one of these flasks was cultivated for the next 4 days at the suboptimal growth temperature, and the other, at the supraoptimal growth temperature.

The dry weight of the algal biomass was determined in the following way: Culture samples were filtered through Vladipor 0.85–0.95-μm-pore-size filters, which were preliminarily dried to complete desiccation and weighed to an accuracy of 0.1 mg. The filters with the algal biomass were dried again to complete desiccation at 70°C and weighed. The dry biomass weight was determined using the difference between the filter weights before and after filtration.

Fatty acid analysis. To analyze intracellular FAs, culture samples of 10–30 ml in volume were filtered through washed Vladipor filters coated with a layer of $BaSO_4$. After partial drying, the $BaSO_4$ layer with the algal biomass was scraped from the filter and extracted immediately thereafter using a chloroform–isopropanol (1 : 1, v/v) mixture. The choice of isopropanol for the extraction was due to its inhibitory action on phospholipases and its good extracting ability, close to that of methanol [16]. The extracts were further treated and methylated as described earlier [17].

To determine extracellular FFAs in the culture liquid filtrates, samples of 20–60 ml in volume were acidified to pH 3–4 with diluted sulfuric acid and supplemented with an internal standard, margaric acid (C17:0). Then lipids were extracted, separated by thin-layer chromatography, methylated, and analyzed by gas–liquid chromatography as described previously [17].

All solvents used were preliminarily purified by distillation, so that their analysis showed the presence of only traces of palmitic and stearic fatty acids.

Fatty acid methyl esters (FAMEs) were tentatively identified by comparing their retention times with those of the authentic samples purchased from Sigma (United States) or Serva (Germany). For this purpose, the FAMEs were separated on plates with silica gel impregnated with an $AgNO_3$ solution (8 : 1), which were developed in a hexane–ethyl ether (85 : 15) mixture. The separated FAMEs were analyzed by gas–liquid chromatography.

To determine the position of double bonds in the FAMEs, they were treated with a *N,O*-bistrimethylsilylacetamide–pyridine (2 : 1) mixture at 50°C for 1 h [17]. The trimethylsilyl derivatives of fatty acids were identified using a Finnigan MAT GCO gas chromatograph–mass spectrometer equipped with a (0.25 mm × 30 m) DB-5MS capillary column with the phenylmethylsilicone phase. The carrier gas was helium; the on-column injection was carried out without flow splitting; the oven temperature was raised from 150 to 250°C at a rate of 4°C/min, from 250 to 280°C at a rate of 8°C/min, and then was held at 280°C for 5 min; the transfer line temperature was 275°C; the temperature of the ion source was 200°C; the ionization energy was 70 eV. The mass spectra of the FAMEs and their trimethylsilyl derivatives were compared with those available in the literature and those of the authentic samples. Based on characteristic ion spectra [18], five fatty acids—16 : 1ω7, 16 : 1ω9, 18 : 1ω9, 16 : 2ω6, and 18 : 2ω6—were identified.

RESULTS

The growth of *S. platensis* and *C. vulgaris* at different temperatures. The growth kinetics of both algae at suboptimal, optimal, and supraoptimal temperatures was almost entirely the same (Fig. 1). Statistically significant differences in the fungal biomasses grown at different temperatures were not revealed either. Presumably, such a small deviation (5°C) from the optimal growth temperature is not essential to the algae within the growth period studied (10 days).

The fatty acid composition of *S. platensis* and *C. vulgaris* cells grown at different temperatures. The lipid extracts of cells of both algae were found to contain polar lipids, chlorophylls, carotenoids, and sterols, but not free fatty acids. In addition, the lipids of *C. vulgaris* contained triacylglycerides. The intracellular fatty acids found in *S. platensis* (16 : 0, 18 : 2ω6, 18 : 3ω6, 18 : 1ω9, 16 : 1ω7 and 16 : 1ω9) are typical of this species. The major fatty acids of *C. vulgaris* are 16 : 0, 16 : 2ω6, 16 : 3ω3, 18 : 2ω6 and 18 : 3ω3 (Table 1).

In *S. platensis*, the contents of 16 : 1 (the sum of 16 : 1ω7 and 16 : 1ω9), 18 : 2ω6, and 18 : 3ω6 decreased and the content of C16 : 0 increased when this alga was grown at the supraoptimal temperature (40°C). The ratios of saturated to monoenoic, of monoenoic to polyenoic, and of dienoic to trienoic fatty acids also increased (Table 1).

In *C. vulgaris* cells grown at the supraoptimal temperature (30°C), the content of trienoic acids was smaller and the content of dienoic fatty acids was greater than in the cells grown at lower temperatures, so that the ratio of dienoic to trienoic acids at the supraoptimal temperature was almost five times higher than at the lower temperatures (Table 1). At the same time, the ratios of saturated to monoenoic and of monoenoic to polyenoic fatty acids did not depend on the growth temperature.

The composition of extracellular free fatty acids in the *S. platensis* and *C. vulgaris* cultures grown at different temperatures. The lipid extracts of the culture liquid filtrates of both algae were found to contain hydrocarbons, FFAs, and polar lipids. The FFAs were represented by only saturated and monoenoic fatty acids (Table 2). The culture liquids did not contain the typical polyenoic intracellular FAs of the algae listed above. The content of the odd-carbon FFAs 15 : 0 and 15 : 1 was sufficiently high. However, these fatty acids were omitted in statistical analysis, since they are not typical of algae [2].

The content of the unsaturated acids 16 : 1 and 18 : 1ω9 in the *S. platensis* culture grown at 40°C was higher and that of the saturated short-chain acids 12 : 0 and 14 : 0

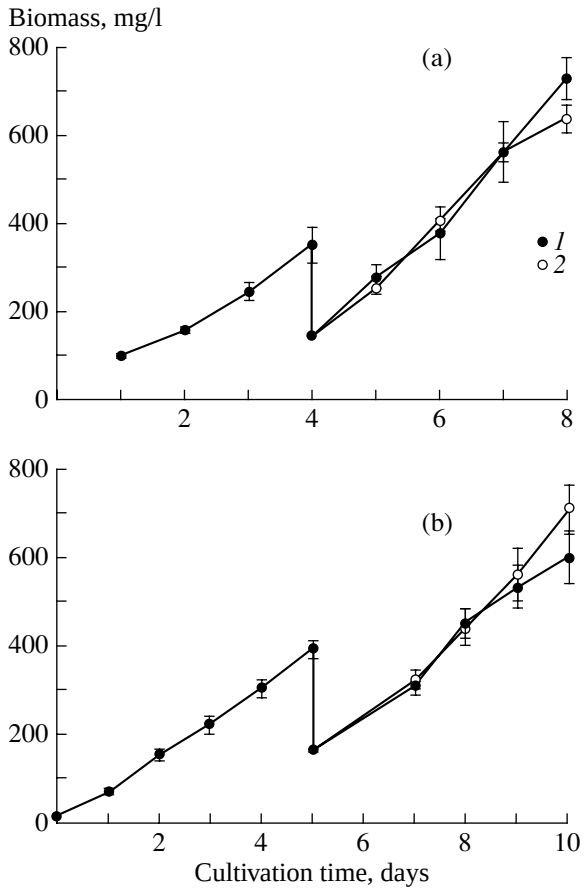

Fig. 1. Dynamics of the biomass (mg/l) of (a) *S. platensis* and (b) *C. vulgaris* grown at different temperatures. Curves *1* (dark circles) show algal growth at the optimal (within 4 or 5 days of cultivation) and suboptimal (after 4 or 5 days of cultivation) temperatures. Curves *2* (open circles) show algal growth at the supraoptimal temperature after 4 (*S. platensis*) or 5 (*C. vulgaris*) days of cultivation.

was lower than in the case of the growth of this alga at 30°C (Table 2). At the same time, the composition of FFAs in the culture liquid of *C. vulgaris* was almost the same at different growth temperatures (Table 2).

The total concentration of unsaturated FFAs (C_{unsat} = 14 : 1ω5 + 16 : 1 + 18 : 1ω9) at elevated and lowered growth temperatures was almost the same in both algae (Table 3). At the same time, the total concentration of extracellular saturated FFAs (C_{sat} = 12 : 0 + 14 : 0 + 16 : 0 + 18 : 0) in the *C. vulgaris* culture grown at the suboptimal temperature was higher than in the case of the growth of this alga at the supraoptimal temperature. The ratio of the total unsaturated to the total saturated FFAs (C_{unsat}/C_{sat}) in the *S. platensis* culture grown at 40°C was higher than in the case of growth at 30°C. In *C. vulgaris* cultures, the ratio C_{unsat}/C_{sat} did not depend on the growth temperature (Table 3).

DISCUSSION

In our experiments, the growth of both algae practically did not depend on temperature (Fig. 1). At the same time, Pinevich *et al.* [14] reported that the growth of *S. platensis* depends on temperature over the same temperature interval that was investigated in the present work (30–40°C). This discrepancy can be explained by the fact that the illumination intensity used in our experiments has a limiting effect on the growth of *S. platensis.* On the other hand, growth temperature considerably influenced the fatty acid composition of the algae. It is tempting to suggest that changes in the fatty acid composition of the algae represent their adaptive response to nonoptimal growth temperatures, which allows the algae to grow normally in the temperature range investigated.

According to some data available in the literature [12, 19], many algae exhibit a general tendency to decrease the degree of unsaturation of fatty acids in response to the elevation of the growth temperature. This tendency may be species-specific. For instance, the increase of the growth temperature of *S. platensis* diminished the desaturation of C16 : 0 into C16 : 1 and of C18 : 1 into C18 : 2 and then into C18 : 3. In the case of *C. vulgaris*, however, growth temperature influenced only the desaturation of the C16 : 2 and C18 : 2 dienoic fatty acids into the respective trienoic fatty acids (Table 1, Fig. 2).

The compositions of extracellular FFAs and intracellular FAs were not similar (Tables 1, 2). The typical polyenoic fatty acids of *S. platensis* (18 : 2ω6 and 18 : 3ω6) and *C. vulgaris* (16 : 2ω6, 16 : 3ω3, and 18 : 3ω3) cells were not found among the extracellular FFAs of these algae. The content of C14 : 0 and C18 : 0 fatty acids in the culture liquid of *S. platensis* and *C. vulgaris* was considerably higher than in the cells of these algae. Similar results were obtained for algae from other taxa [7, 13]. As a rule, the content of polyenoic fatty acids in the culture liquid is low, if present at all. The difference in the composition of extracellular and intracellular fatty acids suggests that the extracellular fatty acids appear in the medium as a result of secretion rather than of cell lysis [6, 7]. Moreover, there is evidence that this secretion is active.

Estimations showed that both algae accumulated approximately equal biomasses (ca. 0.5 g dry wt/l) and extracellular FFAs (ca. 1.0 mg/l) in the growth media (Fig. 1, Table 3). Taking into account that lipids amount to about 10% of the dry biomass of *S. platensis* and that fatty acids amount to about 30% of the total lipids, the calculated concentration of intracellular FAs must be 15–16 mg/l. Therefore, the extracellular FFAs of *S. platensis* and *C. vulgaris* constitute 10–20% of the intracellular FAs of these algae. These estimates agree well with the rates and proportions of the liberation of organic substances by intact algal cells [8].

Table 1. The relative content of intracellular FAs (as a percent of the total) in the enrichment cultures of *S. platensis* and *C. vulgaris* grown at the suboptimal, optimal, and supraoptimal temperatures for 4 days (the exponential growth phase)

Fatty acid	*S. platensis*			*C. vulgaris*				
	$M \pm m$ 30°C	$M \pm m$ 40°C	*t*	*M* 20°C	*M* 25°C	*M* 30°C	η^2	*m*
14 : 0	0.3 ± 0.04	0.2 ± 0.04	1.0	0.5	0.8	0.4	21.6	5.8
14 : 1				0.8	1.0	1.1	5.0	7.0
15 : 0				1.0	1.2	0.7	25.6	5.5
16 : 0	56.8 ± 7.78	73.0 ± 3.69	1.9	23.2	33.9	23.3	35.4	4.8
16 : 1ω7/ω9	2.7 ± 0.35	0.9 ± 0.10	4.9*	1.3	2.0	3.1	61.3**	2.9
16 : 1ω13*tr*				3.0	2.4	1.2	40.7	4.4
16 : 2ω6				5.2	6.2	13.5	84.2**	1.2
16 : 3ω3				18.2	12.5	9.3	68.0**	2.4
18 : 0	1.6 ± 0.43	2.3 ± 0.41	1.3	1.5	2.9	1.2	45.8	4.0
18 : 1ω9	4.5 ± 0.64	4.6 ± 0.39	0.1	2.4	3.3	5.0	55.4**	3.3
18 : 2ω6	14.9 ± 2.76	8.8 ± 1.39	2.0	14.3	16.6	28.1	79.7**	1.5
α-18 : 3				24.6	14.5	11.4	77.2**	1.7
γ-18 : 2	19.4 ± 5.44	10.3 ± 2.02	1.6					
$\frac{\text{SFA}}{\text{UFA}}$	1.42	3.08		0.36	0.62	0.34		
$\frac{\text{MUFA}}{\text{PUFA}}$	0.21	0.29		0.12	0.17	0.17		
$\frac{\text{DUFA}}{\text{TEFA}}$	0.77	0.85		0.44	0.81	2.00		

Note: *M* is the mean value; *m* is the standard deviation (for *C. vulgaris*, *m* was calculated on 2 and 27 degrees of freedom); *t* is Student's statistic calculated for *S. platensis* grown at 30°C ($n = 5$) and 40°C ($n = 6$); η^2 is the influence index of the generalized factor. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DEFA, dienoic fatty acids; and TEFA, trienoic fatty acids.
* $p < 0.05$; ** $\eta^2 > 50\%$.

The maximum effect of growth temperature on the fatty acid composition of *C. vulgaris* cells was observed with respect to polyenoic acids (presumably due to changes in the activity of Δ13 and Δ15 desaturases), whereas the content of intracellular monoenoic acids depended only insignificantly on the growth temperature (Table 1, Fig. 2). The effect of the growth temperature on the content of extracellular monoenoic acids was also weak (Table 2). It is known that polyenoic acids, especially of the ω3 family, are necessary components of the galactolipids of photosynthesizing membranes [2]. It is likely that the observed changes in the content of the ω3 fatty acids of *C. vulgaris* are related to the adaptation of the photosynthesizing membranes of chloroplasts to temperature conditions. This suggestion is confirmed by considerable temperature-induced changes in the content of C16 polyenoic fatty acids, which are primarily synthesized in the chloroplasts.

There is little literature data on the effect of growth temperature on the composition of extracellular and intracellular algal FAs. For instance, Parrish *et al.* [13] showed that elevating the growth temperature for the alga *Gyrodinium aureolum* led to a decrease in the content of highly unsaturated extracellular fatty acids and to a considerable decrease in the content of the intracellular fatty acid 22 : 6ω3. The discrepancy between these data and ours may be due to the fact that Parrish *et al.* investigated total extracellular FAs, whereas we investigated free extracellular FAs.

Thus, we found that the growth temperature considerably influences the fatty acid composition of the investigated algae, both eukaryotic and prokaryotic. With increasing growth temperature, the degree of unsaturation of the intracellular FAs of both algae decreases. The cultivation of the prokaryotic alga *S. platensis* at the supraoptimal temperature led to a decrease in the content of almost all unsaturated fatty acids and to an increase in the content of extracellular monoenoic FFAs. The temperature-induced response of the eukaryotic alga *C. vulgaris* involved changes in the balance between dienoic and trienoic fatty acids. Unlike in the *S. platensis* culture, the composition of

Table 2. The relative content of extracellular FAs (as a percent of the total) in the culture liquids of *S. platensis* and *C. vulgaris* grown at the suboptimal and supraoptimal temperatures for 4 days (the exponential growth phase)

Fatty acid	*S. platensis*			*C. vulgaris*		
	$M \pm m$ 30°C	$M \pm m$ 40°C	$t_{30–40°C}$	$M \pm m$ 20°C	$M \pm m$ 30°C	$t_{20–30°C}$
12 : 0	1.4 ± 0.26	0.6 ± 0.23	2.2*	0.2 ± 0.11	0.1 ± 0.09	0.8
13 : 0	1.4 ± 0.20	0.7 ± 0.16	2.8*	0.1 ± 0.07	0.0 ± 0.01	1.7
14 : 0	8.5 ± 0.56	5.9 ± 0.49	3.5*	4.7 ± 0.49	4.5 ± 0.51	0.4
14 : 1ω5	0.8 ± 0.23	0.8 ± 0.13	0.0	0.9 ± 0.21	0.5 ± 0.19	1.3
15 : 0	5.5 ± 0.44	4.7 ± 0.23	1.6	2.9 ± 0.34	3.0 ± 0.37	0.3
15 : 1	0.3 ± 0.11	0.4 ± 0.14	0.6	0.5 ± 0.15	0.2 ± 0.12	1.1
16 : 0	43.2 ± 1.77	40.1 ± 2.03	1.2	40.6 ± 1.66	42.8 ± 1.51	1.0
16 : 1ω7	4.6 ± 0.78	6.0 ± 0.66	1.3	6.2 ± 0.87	5.8 ± 0.40	0.5
*i*17 : 0	0.0	0.1 ± 0.10	1.0	0.1 ± 0.08	0.1 ± 0.12	0.0
18 : 0	30.5 ± 2.12	3.33 ± 1.95	1.0	36.0 ± 2.82	35.7 ± 1.73	0.1
18 : 1ω9	3.8 ± 0.84	7.5 ± 1.02	2.8*	7.7 ± 0.87	7.2 ± 0.71	0.5

Note: *M* is the mean value; *m* is the standard deviation; *t* is Student's statistic calculated for *S. platensis* grown at 30°C ($n = 10$) and 40°C ($n = 12$) and for *C. vulgaris* grown at 20°C ($n = 12$) and 30°C ($n = 11$).
* $p < 0.05$.

Table 3. The concentrations (mg/l) of saturated (C12 : 0 + C14 : 0 + C16 : 0 + C18 : 0) and unsaturated (C14 : 1ω5 + C16 : 1 + C18 : 1ω9) FFAs and their proportions (C_{unsat}/C_{sat}) in the culture liquids of *S. platensis* and *C. vulgaris* grown at the suboptimal and supraoptimal temperatures

Days of cultivation	*S. platensis*						*C. vulgaris*					
	C_{sat}		C_{unsat}		C_{unsat}/C_{sat}		C_{sat}		C_{unsat}		C_{unsat}/C_{sat}	
	30°C	40°C	30°C	40°C	30°C	40°C	20°C	30°C	20°C	30°C	20°C	30°C
5 (7)	0.174	0.205	0.011	0.033	0.064	0.160	1.160	0.605	0.191	0.193	0.191	0.193
5 (7)	0.159	0.104	0.004	0.010	0.027	0.097	0.508	0.487	0.160	0.158	0.160	0.158
5 (7)		0.085		0.010		0.115	0.386	0.497	0.139	0.112	0.139	0.112
6 (8)	0.188	0.129	0.026	0.022	0.137	0.171	0.951	1.006	0.310	0.221	0.310	0.221
6 (8)	0.237	0.148	0.048	0.033	0.201	0.224	1.040	0.996	0.211	0.172	0.211	0.172
6 (8)	0.164	0.107	0.021	0.013	0.130	0.118	0.914	0.469	0.122	0.121	0.122	0.121
7 (9)	0.171	0.184	0.012	0.024	0.070	0.133	1.241	0.684	0.174	0.155	0.174	0.155
7 (9)	0.134	0.284	0.007	0.031	0.049	0.107	0.968	0.653	0.340	0.200	0.340	0.200
7 (9)	0.188	0.247	0.015	0.039	0.078	0.157	0.794	0.613	0.208	0.199	0.208	0.199
8 (10)	0.268	0.335	0.068	0.098	0.254	0.292	0.703	0.872	0.114	0.108	0.114	0.108
8 (10)	0.296	0.322	0.038	0.094	0.129	0.293	1.001	0.715	0.094	0.161	0.094	0.161
8 (10)		0.235		0.076		0.322	0.763		0.166		0.166	
M	0.198	0.199	0.025	0.040	0.114	0.182	0.869	0.608	0.166	0.115	0.186	0.164
m	0.016	0.025	0.006	0.009	0.022	0.023	0.073	0.070	0.026	0.015	0.022	0.015
CV (%)	8.4	12.6	26.0	22.5	19.8	12.7	8.4	11.6	15.6	12.8	11.6	7.0
t	0.03		1.37		2.10*		2.58*		1.71		0.83	

Note: *M* is the mean value; *m* is the standard deviation; *t* is Student's statistic; and *CV* is the coefficient of variation.

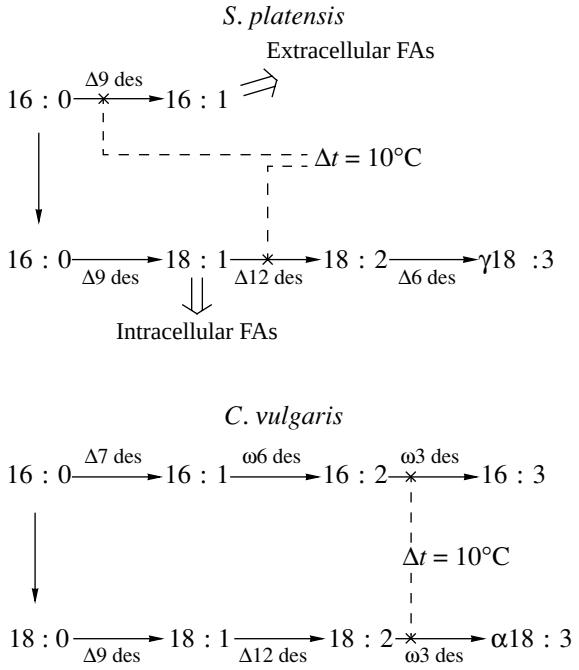

Fig. 2. Putative pathways of fatty acid biosynthesis in *S. platensis* and *C. vulgaris.* ω and Δ indicate the position of the first double bond beginning with, respectively, the methyl and carboxyl termini of the FA molecules.

extracellular FFAs in the *C. vulgaris* culture was practically independent on the growth temperature.

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REFERENCES

1. Kenyon, C.N., Rippka, R., and Stanier, R., Fatty Acid Composition and Physiological Properties of Some Filamentous Blue–Green Algae, *Arch. Microbiol.*, 1972, vol. 83, pp. 216–236.
2. Cobalas, M.A. and Lechado, J.Z., Lipids in Microalgae: A Review I. Biochemistry, *Grasas y Aceites* (Esp.), 1989, vol. 40, pp. 118–145.
3. Vestal, J.R. and White, D.C., Lipid Analysis in Microbial Ecology: Quantitative Approaches to the Study of Microbial Communities, *BioSci.*, 1989, vol. 39, pp. 535–540.
4. Reemtsma, T., Haake, B., Ittekkot, V., Nair, R., and Brockmann, U., Downward Flux of Particulate Fatty Acids in the Central Arabian Sea, *Mar. Chem.*, 1990, vol. 29, pp. 183–202.
5. Gladyshev, M.I., Sushchik, N.N., Kalacheva, G.S., and Shchur, L.A., The Composition of Free Fatty Acids Present in the Surface Water Film and the Kinetics of Self-Cleaning from Phenol in a Forest Pond during the *Volvox aureus* Bloom, *Dokl. Akad. Nauk*, 1996, vol. 349, no. 6, pp. 840–843.
6. Gladyshev, M.I., Kalachova, G.S., and Sushchik, N.N., Free Fatty Acids of Surface Film of Water in the Sydinsky Bay of the Krasnoyarsk Reservoir, *Int. Rev. Ges. Hydrobiol.*, 1993, vol. 78, no. 4, pp. 575–578.
7. Billmire, E. and Aaronson, S., The Secretion of Lipids by the Freshwater Phytoflagellate *Ochromonas danica, Limnol. Oceanogr.*, 1976, vol. 21, no. 1, pp. 138–140.
8. Fogg, G.E., The Ecological Significance of Extracellular Products of Phytoplankton Photosynthesis, *Bot. Mar.*, 1983, vol. 25, pp. 3–14.
9. Staats, N., De Winder, B., Stal, L.J., and Muur, L.R., Isolation and Characterization of Extracellular Polysaccharides from Epipelic Diatoms *Cylindrotheca closterium* and *Navicula salinarium, Eur. J. Phycol.*, 1999, vol. 34, pp. 161–169.
10. Parrish, C.C., Dissolved and Particulate Marine Lipid Classes: A Review, *Mar. Chem.*, 1988, vol. 23, pp. 17–40.
11. Meyers, A.P. and Kawka, O.E., Fractionation of Hydrophobic Organic Materials in Surface Microlayers, *J. Great Lakes Res.*, 1982, vol. 8, no. 2, pp. 288–298.
12. Harwood, J.L. and Jones, A.L., Lipid Metabolism in Algae, *Advances in Botanical Research*, Callow, J.A., Ed., London: Academic, 1989, vol. 16, pp. 1–53.
13. Parrish, C.C., Bodennec, G., Sebedio, J.-L., and Gentien, P., Intra- and Extracellular Lipids in Cultures of the Toxic Dinoflagellate *Gyrodinium aureolum, Phytochemistry*, 1993, vol. 32, pp. 291–295.
14. Pinevich, V.V., Verzilin, N.N., and Mikhailov, A.A., Investigation of *Spirulina platensis*, a Novel Subject for Intense Cultivation, *Fiziol. Rast.*, 1970, vol. 17, no. 5, pp. 1037–1046.
15. Sirenko, L.A., A Technique for the Laboratory Cultivation of Algae, in *Metody fiziologo-biokhimicheskogo issledovaniya vodoroslei v gidrobiologicheskoi praktike* (Physiological and Biochemical Methods in the Hydrobiological Investigation of Algae), Kiev: Nauk. Dumka, 1975.
16. *Lipids. CRC Handbook of Chromatography*, Mangold, H.K., Ed., Florida: CRC, 1984, vol. 1, pp. 33–46.
17. Kalacheva, G.S. and Sushchik, N.N., The Fatty Acid Composition of *Spirulina platensis* as a Function of Its Age and Mineral Nutrition, *Fiziol. Rast.*, 1994, vol. 41, no. 2, pp. 275–282.
18. Capella, P. and Zorzut, C.M., Determination of Double Bond Position in Monounsaturated Fatty Acid Esters by the Mass Spectrometry of Their Trimethylsilyloxy Derivatives, *Anal. Chem.*, 1968, vol. 40, pp. 1459–1461.
19. Cohen, Z., Vonshak, A., and Richmond, A., Fatty Acids Composition of *Spirulina platensis* Grown under Various Environmental Conditions, *Phytochemistry*, 1987, vol. 26, pp. 2255–2258.