

Comparative Investigation of Fatty Acid Composition in Lipids of Various Strains of *Porphyridium cruentum* and *Porphyridium aerugineum*

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Abstract—The comparative study of the fatty acid composition of seven strains of *Porphyridium cruentum* and four strains of *Porphyridium aerugineum* was carried out by using GLC and mass-spectrometry methods. The presence of arachidonic and eicosapentaenoic acids was detected in all strains studied, both “freshwater” *P. aerugineum* and “marine” *P. cruentum*. At equal light intensity and suspension density, the strains of *P. aerugineum* compared to those of *P. cruentum* contained more eicosapentaenoic and *trans*-3-hexadecenoic acids, which are the constituents of thylakoid membrane lipids. With an increase in suspension density upon culture transition to the stationary growth phase (cessation of cell division and cell enlargement), the content of eicosapentaenoic and *trans*-3-hexadecenoic acids decreased, while the content of arachidonic, eicosatrienoic, and 18-C acids became somewhat higher. The extent of these alterations was related both to the strain and species specificity and to cell age. The species specificity for fatty acid composition was more distinct during the stationary phase. In addition, the accumulation of different intermediate products of polyunsaturated fatty acid (PUFA) biosynthesis in both species during culture aging allow us to suggest that there are alternative pathways of PUFA biosynthesis.

Key words: *Porphyridium* strains - growth phases - arachidonic acid - eicosapentaenoic acid

INTRODUCTION

The genus *Porphyridium* (class Bangiophyceae, order Porphyridiales), unlike the majority of red algae, includes unicellular algae adapted to living in temperate-zone waters varying in salinity (from fresh water to sea water) or as colonies on soil and other solid substrates. The individual species of *Porphyridium* adapted to different habitats can vary significantly in their metabolism, the most prominent feature being diverse pigment composition. Among the accessory pigments, phycoerythrins prevail in *P. cruentum*, and phycocyanin is most abundant in *P. aerugineum* [1 - 3]. The presence of 20-C PUFA is characteristic of *P. cruentum* lipids. Among these acids, the major ones are eicosapentaenoic acid located in galactolipids of thylakoid membrane and arachidonic acid as a component of phospholipids and triacylglycerols [1, 4, 5]. The dependence of fatty acid content and composition on the culture-growth phase, light intensity, temperature, mineral nutrition, medium salinity, and on effects of detergents was demonstrated in a number of works [6 - 10]. Most of these studies dealt with *P. cruentum*

Vischer 107 and ignored other collection strains. There is little information about the lipids of *P. aerugineum*. In particular, the data concerning the presence of 20-C PUFA in this alga is discrepant [11, 12].

As 20-C PUFA are assumed to be mainly confined to marine organisms [1, 12, 13], and there is only poor information about the fatty acids of *P. aerugineum* inhabiting fresh or low-salinity waters, it was of interest to study the fatty acids in various strains of this species in comparison to some strains of *P. cruentum*. It appeared important to elucidate whether the freshwater red algae synthesize 20-C PUFA and to estimate variability in the fatty acid composition among the strains of each species.

MATERIALS AND METHODS

The unicellular red alga *Porphyridium cruentum* Nag. (*P. purpureum* Bory. Drev. et Ros) st. 2, st. 3, st. 4, st. 5, st. 6, st. 7, and st. 12 and *P. aerugineum* Geitl. st. 9, st. 10, st. 11, and st. 13 were used. All strains were taken from the working collection of the Institute of Microbiology, Czech Academy of Sciences. The *P. cruentum* st. 2 and st. 3 correspond to *P. cruentum* st. 1380-1b and st. 1380-1c in terms of the Schlösser

Abbreviations: MGDG - monogalactosyl diacylglycerols; PUFA - polyunsaturated fatty acids; st. - strain

collection [14]. Both strains were initially isolated from non-sea habitats, but they were maintained in collections on a media of high salinity for over three decades. Other strains of *P. cruentum* studied were either marine organisms, or were harvested from the soil near sea shores. Among the strains of *P. aeruginum*, st. 9 and st. 11 are freshwater forms, and st. 10 was initially isolated from low-salinity waters (in the Schlösser nomenclature, they are designated as *P. aeruginum* st. 1380-2, st. B 110.79, and st. B 111.79, respectively). In relation to salinity, these two species are designated as "marine" and "freshwater" algae, which appears reasonable regarding their origin. The most frequently studied strain, *P. cruentum* Vischer 107, was not used in this work, but it was studied earlier under identical conditions [5, 15]. Moreover, its properties were well known from other works [7, 9] and used for comparison in present study.

The algae were grown as a batch culture in tubes in conditions of constant bubbling through suspensions with a gas mixture (air + 2% CO₂), at an illumination of 40 W/m², and at 25°C. The Brody-Emerson medium, supplemented with microelements in accordance with Setlik (see [15, 16]), was used for *P. cruentum* cultivation; the Ramus medium [17] with the same microelement set was used for the *P. aeruginum* culture. The samples at the linear growth phase were done in roughly the same ranges of optical density level for all strains. The samples were also harvested at culture transition to the stationary phase. The cells were harvested by centrifugation. The culture growth was monitored by the optical density of the suspensions, the number of cells, and biomass accumulation. The culture state was also inferred from the content and composition of pigments, using a spectrophotometer (Shimadzu, Japan) [15, 18].

The *Porphyridium* cultures accumulate not only cell biomass, but also some extracellular substances, first of all, some exopolysaccharides [7, 17]. Their amounts were of importance for evaluating the culture production. For the total biomass estimation, exopolysaccharides were precipitated with ethanol, and the total weight of the pellet, including the cells and exopolysaccharides, was measured after drying at 105°C. However, for all calculations in this work, only the cell dry weight values, without correction for extracellular components, were used.

For lipid extraction, 30 ml of distilled water was added to 40 ml of algal suspension and centrifuged at 1400 g for 20 min. The pellet was rinsed with a 0.2% NaCl solution. Then the water phase was carefully drawn off and 10 ml of hot methanol with a pinch of ionol as an antioxidant was added to the pellet. After a 3-min boiling and subsequent cooling, the mixture was supplemented with 20 ml of chloroform and 10 ml of water and centrifuged at 1400 g for 5 min for phase separation. The lower layer was sucked off with a syringe. The upper layer was repeatedly washed with chloroform, and all chloroform fractions were combined and vacuum evaporated. The residue was dried in

a desiccator, weighed, resuspended in benzene, and transferred into an ampulla to be sealed up under nitrogen and preserved at -20°C until used for analysis.

Prior to analysis for fatty acids, their methyl esters were obtained by methanolysis of the lipid extract in a 5% acetyl chloride-methanol at 65°C for 50 min. The methyl esters were then purified by TLC carried out on plates with a layer of L 5/40 μm silica gel (Chemapol, Czech Republic) supplemented with 1% gypsum; benzene was used as a mobile phase. The methyl esters of fatty acids were examined with a Chrom 5 chromatograph equipped with a C1-100 integrator (Laboratory pristroje, Czech Republic). Polyethyleneglycol adipate (PEGA) (Reakhim, USSR) on celite 545 was used for column package, and nitrogen was used as a carrier gas (1.6 × 10 Pa). The temperature in the thermostat was 196°C, and that in the injection chamber was 220°C. The identification of fatty acids was performed in reference to their relative retention times in comparison with those of authentic samples. For acid quantitation, margaric (C17 : 0) acid was used as an internal standard. Each value was the mean for 2 to 3 samples (from 4 to 6 chromatograms). The standard errors were not greater than 5% for major acids and 10% for minor components. Some samples were additionally analyzed by GLC using an open-tube columns with Silar 10C and OV-17 (Serva, Germany) liquid phases. Gas chromatomass spectrometry was employed as well. The analyses were performed on an LKB GC-MS 2091 installation (LKB, Sweden). The procedure was carried out using a packed column (Silar 10C), at a constant temperature of 165°C. Mass-spectra were obtained at an ionization voltage of 70 and 20 eV.

RESULTS

The study of related organisms adapted to different conditions always raises the question of whether it is better to compare them under identical conditions or under the conditions most favorable to each. Two *Porphyridium* species (*P. cruentum* and *P. aeruginum*) are distinguished significantly due at least to the different composition of the accessory pigments [1 - 3]. This leads to qualitative differences with respect to light absorption even under the same light source (Fig. 1). Furthermore, the strains of these two species were adapted in collections to different culture media. Thus, by providing for the same temperature, light, and suspension density we can not consider the conditions as equally favorable to both species.

Table 1 shows that the cells of the *P. aeruginum* strains are smaller, on average, than those of *P. cruentum*, though significant variability exists among the strains of each species. Therefore, at the same optical density, the suspensions of the former species had a greater number of cells and lower biomass content than the latter. The total content of fatty acids was rather variable, but its value tended to be higher in the biomass of *P. aeruginum* than in *P. cruentum* (Table 1).

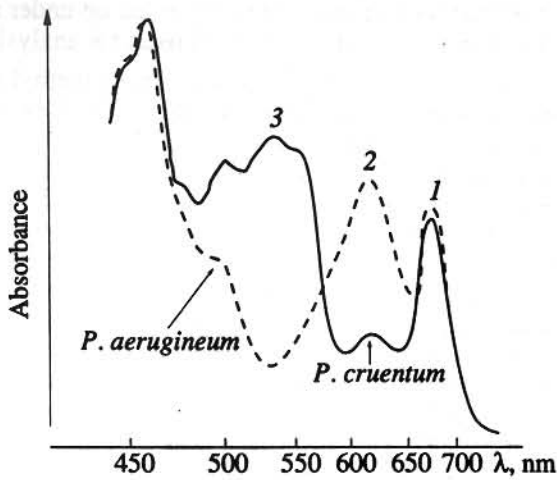


Fig. 1. The absorption spectra of the suspensions of *P. cruentum* and *P. aeruginense*. The bands of absorption: (1) chlorophyll, (2) phycocyanin, and (3) phycoerythrin.

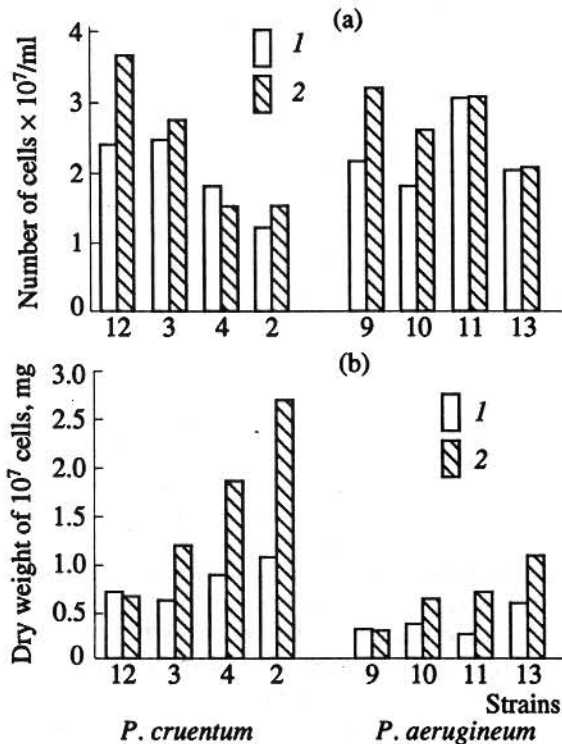


Fig. 2. The number of cells in suspension and the dry weight of cells at (1) the linear and (2) stationary phases of growth in various strains of *P. cruentum* and *P. aeruginense*. (a) the number of cells $\times 10^7$ per 1 ml of suspension, (b) dry weight of 10^7 cells, mg.

The investigation of fatty acid composition detected 20-C PUFA in all strains of both *P. cruentum* and *P. aeruginense*. The presence of eicosapentaenoic and arachidonic acids was proven by the GLC and MS for all strains of *P. aeruginense* (Table 2). This implied that many strains of this species had long-chain PUFA in spite of the fact that they inhabit fresh or low-salinity

water. In this experiment, a higher content of C20 : 3n3 and C20 : 5n3 and a relatively low content of C20 : 4n6 was found for *P. aeruginense* (in three strains of four). Concerning the content of other acids, variability among the strains of each species was not less than that between the two species. This variability appears to be dependent not only on the genetic organization of alga, but on its physiological state as well.

To evaluate potential variabilities in fatty acid composition in relation to the physiological state of the algae, the cultures were analyzed not only during the linear phase, but also at transition to the stationary phase and for some period of their staying on the "plateau." On a culture transition to the stationary phase, the cells cease to divide and the proportion of large cells usually increases; this is typical for many algal species [1, 6, 19]. In our experiments (Fig. 2), the various strains showed different extents of approaching the "plateau" that unequivocally affected fatty acid content. In both *P. cruentum* and *P. aeruginense* species, while some strains only approached the stationary phase (st. 9 and st. 12), the cells did not change size yet continued to divide; the other strains had slower dividing cells of enlarged size (st. 3 and st. 10). At last, for some strains on the "plateau," no cell division was noted, and the cells were significantly enlarged (st. 4, st. 11, and st. 13).

Table 3 shows the fatty acid composition of lipids for two strains belonging to *P. cruentum* (st. 3) and *P. aeruginense* (st. 10). Some decrease in the total content of fatty acids on a dry weight basis could result from the accumulation of carbohydrates in the cells during culture aging [17, 20]. The increase in the fatty acid content calculated on a cell basis was possibly related to cell enlargement. In the cells of both species, the absolute and relative contents of C16 : 1, C16 : 1n13tr, and C20 : 5n3 decreased, but those of C18 : 0, C18 : 1n9, C18 : 2n6, and C20 : 4n6 increased. In this case, the increase in the content of 18-C acids and the decrease in C20 : 5n3 content was more pronounced in the *P. cruentum* strain than in the *P. aeruginense* strain. Analogous changes in fatty acid content had been observed earlier on aging cultures of *Porphyridium* [6, 9, 21] and on some other algae [1, 20, 22]. In addition, the increase in the absolute and relative contents of C20 : 3n6 in *P. cruentum* (st. 3) and C20 : 3n3 in *P. aeruginense* (st. 10) is worth mentioning.

Table 4 presents data on the ratios of the content of total and individual fatty acids in the biomass at the stationary phase of growth to those values obtained for the linear phase. The calculation of these ratios was based on results similar to those presented in Table 3 for two representative strains. As the scope of this paper prevents us from presenting all our results, Table 4 shows only the values related to fatty acids calculated on a dry weight basis. The data indicate that the differences between the *P. cruentum*, st. 3 and *P. aeruginense*, st. 10, are also valid for other strains of these two *Porphyridium* species. All strains, even those exhibiting the very beginning of the transition to the stationary phase

(*P. cruentum* st. 12 and *P. aeruginosa* st. 9) manifested changes typical for both species (an increase in the content of C18 : 0, C18 : 1n9, and C20 : 4n6) and specific for each of them (a decrease in C20 : 5n3 and an increase in C18 : 2n6, C18 : 3n6, C20 : 2, and C20 : 3n6 for *P. cruentum*, or C20 : 3n3 increase for *P. aeruginosa*). It should be noted that the decrease in C16 : 1n13tr concentration (mol %) was not observed in st. 9 and st. 12 as it was in the cultures more advanced in aging. The changes in proportions of all other acids (mol %) corresponded to those in the typical strains (see Table 3).

As changes in the content and proportion of definite PUFAs exhibited opposite trends, their ratios changed considerably at transition to the stationary phase: the ratio of C20 : 5n3 to C20 : 4n6 or to C18 : 2n6 declined (Fig. 3). These ratios correlated with changes in cell weights suggesting that genetic traits alone are hardly responsible for interspecies differences.

DISCUSSION

The presence of 20-C PUFA is a characteristic feature of sea organisms, including algae, which serve as the first members in many trophic chains [1, 12, 13].

The problem of the presence of these acids in freshwater organisms seems to be more complicated. Some freshwater algae contain 20-C PUFA, though it is suggested to be an exception rather than the rule [12, 23, 24]. It is not easy to decide if the presence of long-chain PUFA in freshwater or terrestrial organisms reflects their genetic relationships to "marine" ancestors and if the long-chain PUFA can emerge as an adaptive response to certain environmental conditions. We have found that some strains of the freshwater alga *P. aeruginosa* and some strains of *P. cruentum* isolated from low-salinity waters contain long-chain PUFA (C20 : 4n6 and C20 : 5n3), as do the true sea algae. In this case, the algae exhibited the properties of their marine ancestors, in spite of living in freshwater.

Up to now, the content and composition of fatty acids are not employed as a conventional criterion for indicating relationships between organisms: they are equivocal, and physiological variability causes trouble while interpreting results. However, in recent years, this problem has attracted more and more attention [22, 24 - 27]. Moreover, some qualitative differences in the fatty acid composition of closely related organisms are shown to indicate some imperfections in their classification. For example, the presence of C16 : 4 in *Chlorella fusca*, absent from other *Chlorella* species, and some peculiarities in the ultrastructure and chemical composition, necessitated the exclusion of *C. fusca* from the genus *Chlorella* [28].

In this work, the two *Porphyridium* species significantly differed both in growth (Table 1) and in the content and composition of fatty acids (Table 2). However, it is difficult to conclude if the higher content of C16 : 1n13tr and C20 : 5n3 in *P. aeruginosa* is a spe-

Table 1. Growth characteristics and fatty acid content in the cells of *P. cruentum* and *P. aeruginosa* at the linear phase of culture growth

Strains	Biomass, mg/ml	Cell number × 10 ⁷ /ml	Dry wt, of 10 ⁷ cells, mg	Fatty acids, % of dry wt
<i>P. cruentum</i>				
2	1.27	1.19	1.07	4.15
3	1.53	2.46	0.62	4.63
4	1.58	1.79	0.88	4.49
5	1.42	1.14	1.25	5.60
6	1.11	0.96	1.18	8.18
7	1.27	1.12	1.34	6.27
12	1.64	2.38	0.69	4.77
<i>M</i> *	1.44	1.58	1.05	5.44
<i>m</i> **	0.20	0.63	0.34	1.41
<i>P. aeruginosa</i>				
9	0.66	2.12	0.31	7.41
10	0.62	1.75	0.36	9.47
11	0.73	2.99	0.24	7.01
13	1.11	1.97	0.56	6.04
<i>M</i>	0.78	2.21	0.38	7.48
<i>m</i>	0.22	0.54	0.14	1.44
<i>P. cruentum</i> / <i>P. aeruginosa</i>	1.79	0.72	2.76	0.72

Notes: * *M* - means.

** *m* - standard errors.

cies-specific property or if it relates to the age structure of the population, light distribution in the suspension, or the spectral composition of the absorbed light, as the correlation of all these parameters with fatty acid composition is well documented [6, 9, 15, 21].

The species-specific properties manifested themselves more distinctly after culture transition to the stationary phase. At such transition, the content of C20 : 5n3 decreased and that of C20 : 4n6 increased in all strains. The content of C18 : 0, C18 : 1n9, C18 : 2n6, and eicosatrienoic acids, as the intermediate products of long-chain PUFA biosynthesis [1, 12, 13, 29] also increased. This resulted in a decrease in the ratio of both eicosapentaenoic to arachidonic and eicosapentaenoic to linoleic acid (Fig. 3). Eicosapentaenoic acid is located in galactolipids of thylakoid membranes, mainly in MGDG, and it is assumed to play the same role there as C18 : 3n3 played in the chloroplasts of higher plants and green algae [1, 4, 5]. Similar changes in the ratios of most unsaturated fatty acid to their biosynthetic precursors have been shown earlier for some algae as to be an adaptive response of the

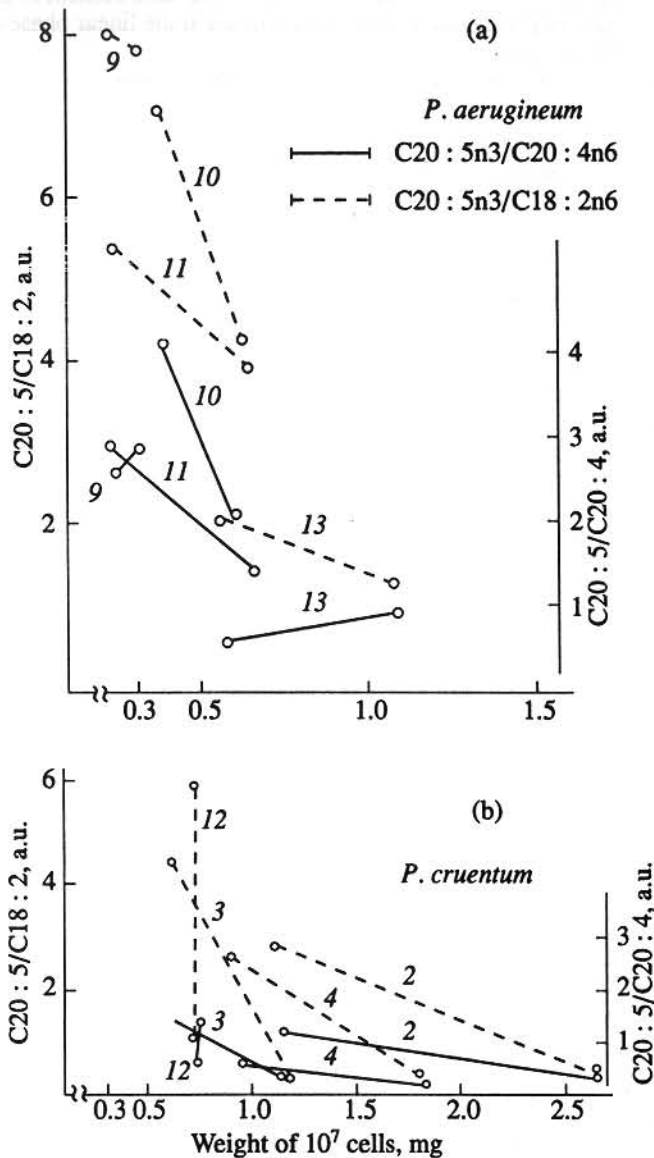


Fig. 3. The cell weight-dependent changes in the ratio of PUFA in various strains of *P. aeruginosa* and *P. cruentum*. The collection number of each strain is designated on the figure. The increase in cell weight is related to culture transition to the stationary phase (see Fig. 2).

photosynthetic apparatus to environmental changes [30, 31]. This can also reflect an attenuating of photosynthetic activity with age related to the suppression of processes of fatty acid elongation and desaturation [1, 12, 13, 29, 30]. We and other authors observed similar phenomena earlier in the experiments with *P. cruentum* Vischer 107 [8, 9, 15, 21, 32]. At the same time, the increase in C18:2n6 and C20:4n6 could depend on storage lipid accumulation under unfavorable conditions as those acids are the major components of triacylglycerols in the *Porphyridium* cells [4, 5].

As for comparative studies of different strains of a species, difficulties in interpreting results are even greater than for different species of the same genus. In

our experiments, the st. 13 of *P. aeruginosa* was highly distinctive from other strains of this species, resembling the strains of *P. cruentum* in many of its properties (Tables 1 and 2). There were only insignificant differences among the strains of *P. cruentum*, mainly concerned with the content of minor components. At the stationary phase of cell growth, the culture age, rather than genetic traits, influenced strain properties.

In recent studies, Cohen *et al.* [32] compared the content of individual fatty acids at the linear and stationary phase of culture growth; many strains of *P. cruentum* were identical to those in our experiments but had a somewhat higher content of C20:5n3 and a lower content of C16:0, which gave suggested more favorable conditions for photosynthesis in that work [32]. Nevertheless, at the transition to the stationary phase, the culture grown at 25°C manifested, though to a lesser extent, the same regularities as those in our study. At a temperature increase up to 30°C, the fatty acid composition changes were similar and even more pronounced. Thus, the response to unfavorable conditions was independent of whether it was culture aging or high temperature.

All strains in our experiments manifested common trends in the change of their fatty acid composition at transition to the stationary phase, but the content of C18:2n6 and C20:3n6 was higher in *P. cruentum*, whereas C20:3n3 concentration was much more great augmented in *P. aeruginosa*. These fatty acids are considered to be intermediates on the pathway of C20:4n6 and C20:5n3 biosynthesis, i.e., the biosynthesis of most unsaturated fatty acids inherent to this alga. There now exists a generally accepted scheme for the biosynthetic pathway of arachidonic, but not for eicosapentaenoic acid, the latter being less studied [1, 4, 13, 29]. The divergence of the pathways of these acids is assumed to occur at some early stage, namely, at the level of C18:2n6 transformation. Keeping in mind that the biosynthesis of eicosapentaenoic acid is reduced during culture growth deceleration in all strains and considering the findings in terms of the accepted scheme of PUFA biosynthesis, we can suggest the following. In *P. aeruginosa*, one of the last stages of C20:5n3 biosynthesis, namely the reaction of C20:5n3 dehydrogenation, is most severely blocked. In the strains of *P. cruentum*, an earlier stage of biosynthesis, perhaps at the level of the divergence of the n6- and n3-pathways, is most inhibited. As a result, the accumulation of n6-acids occurred. However, it is possible that the transition to the biosynthesis of n3-acids can proceed at some later stage, not at the level of C18:2n6 dehydrogenation. The presence of C20:3n6 in MGDG and the dynamics of fatty acid content in various lipid groups in *Porphyridium* under environmental changes [21, 32] confirm this suggestion. This is in line with the conclusion that C20:2n6 is an intermediate of arachidonic acid biosynthesis in the fungus *Saprolegnia* [33] and in *Euglena gracilis* [4], whereas, in *P. cruentum* and in *Ochromonas danica*, the biosynthesis of arachidonic acid proceeds via C18:3n6 [4].

Table 2. Lipid fatty acid composition of *P. cruentum* and *P. aeruginosa* cultures at the linear phase of growth, mol %

Fatty acids	<i>P. cruentum</i> , Strains							<i>P. aeruginosa</i> , Strains			
	4	6	5	7	2	12	3	13	11	9	10
C16:0	44.23	51.66	45.8	50.37	37.23	44.5	40.62	40.85	55.44	54.7	45.82
C16:1n9	2.14	1.08	1.2	0.67	3.57	1.26	1.31	1.39	1.18	1.1	3.21
C16:1n13tr	1.51	1.12	1.53	1.05	1.87	1.71	1.98	1.65	2.32	0.9	2.8
C18:0	0.85	0.36	0.44	0.5	1.29	0.67	1.18	1.2	0.46	0.9	0.45
C18:1n9	0.91	0.39	0.43	0.43	0.87	0.66	0.78	0.63	0.59	1.1	0.6
C18:2n6	5.7	4.63	5.49	7.02	7.41	4.4	5.8	4.07	4.49	3.3	4.73
C18:3n6	0.38	0.21	0.16	0.24	0.57	0.22	0.4	0.38	0.32	0.33	0.24
C20:2	0.72	0.35	0.43	0.27	1.25	0.49	0.63	1.36	0.23	0.7	0.6
C20:3n6	0.51	0.3	0.24	0.2	0.47	0.41	0.51	3.19	1.86	1.8	0.85
C20:4n6	27.81	24.45	26.35	19.72	19.24	20.1	18.86	27.17	8.18	8.9	7.96
C20:3n3	0.6	0.89	0.53	0.53	3.03	1.04	1.57	2.5	1.07	1.1	tr*
C20:5n3	14.79	14.88	17.28	19.14	23.25	24.64	26.43	15.74	23.35	25.2	32.57
% of dry wt	4.49	8.18	5.6	6.27	4.15	4.77	4.63	6.04	7.01	7.41	9.47

Notes: Fatty acids:

C16:0	palmitic	C18:0	stearic	C20:2**	eicosadienoic
C16:1n9	hexadecenoic	C18:1n9	oleic	C20:3n6	dyhomo- γ -linolenic
C16:1n13tr	trans-3-hexadecenoic	C18:2n6	linoleic	C20:4n6	arachidonic
C16:2*	hexadecadienoic	C18:3n6	γ -linolenic	C20:3n3	eicosatrienoic
		C18:3n3*	α -linolenic	C20:5n3	eicosapentaenoic

* Traces.

** Double bond position is unknown.

Table 3. Fatty acid content and composition in two representative strains of *P. cruentum* and *P. aeruginosa* at the linear (1) and stationary (2) phases of growth

Fatty acids*	<i>P. cruentum</i> , St. 3						<i>P. aeruginosa</i> , St. 10					
	$\mu\text{g}/10\text{ mg}$ of dry wt		$\mu\text{g}/10^8$ cells		mol %		$\mu\text{g}/10\text{ mg}$ of dry wt		$\mu\text{g}/10^8$ cells		mol %	
	1	2	1	2	1	2	1	2	1	2	1	2
C16:0	188.1	130	116.8	155.4	40.62	33.24	433.9	330.4	154.5	206.6	45.82	46.28
C16:1	6.1	1.9	3.8	2.2	1.31	0.48	30.4	5.4	10.8	3.4	3.21	0.76
C16:1n13tr	9.2	4.9	5.7	5.9	1.98	1.26	26.5	14.2	9.4	8.9	2.8	1.99
C18:0	5.5	7.4	3.4	8.8	1.18	1.88	4.3	7.2	1.5	4.5	0.45	1.01
C18:1n9	3.6	18.3	2.2	21.8	0.78	4.67	5.7	9.6	2	6	0.6	1.34
C18:2n6	26.9	73.9	16.7	88.4	5.8	18.9	44.8	42.4	16	26.5	4.73	5.94
C18:3n6	1.9	1.8	1.2	2.2	0.4	0.47	2.3	1.6	0.8	1	0.24	0.22
C20:2	2.9	1.4	1.8	1.7	0.63	0.36	5.7	3.6	2	2.3	0.6	0.51
C20:3n6	2.4	10.4	1.5	12.4	0.51	2.65	8.1	6.9	2.9	4.3	0.85	0.96
C20:4n6	87.3	119.1	54.2	142.3	18.86	30.45	75.4	96	26.8	60	7.96	13.44
C20:3n3	7.1	**	4.3	**	1.51	-	0.3	26.6	0.1	16.6	0.03	3.72
C20:5n3	122.4	22.1	76	26.5	26.43	5.66	308.4	168.4	109.8	105.3	32.57	23.59
Sum	463	391	287.6	467.5	-	-	947	714	337.1	446.3	-	-

Notes: * For fatty acid designations see Table 2.

** Traces.

Table 4. The ratio of fatty acid content on dry weight basis at the linear growth phase to that at the stationary phase

Fatty acids	<i>P. cruentum</i> , Strains				<i>P. aeruginum</i> , Strains		
	12	3	4	2	9	10	11
C16 : 0	0.57	0.69	0.79	0.45	0.95	0.76	0.85
C16 : 1	0.8	0.31	0.37	0.13	0.59	0.18	1.07
C16 : 1n13tr	0.6	0.53	0.44	0.41	1.04	0.54	0.82
C18 : 0	1.31	1.35	2.54	1.09	1.61	1.67	1.53
C18 : 1n9	2.34	5.08	7.01	3.32	1.46	1.68	1.49
C18 : 2n6	1.11	2.75	4.36	1.3	1.09	0.97	1.18
C18 : 3n6	0.91	0.95	3.26	0.61	1.01	0.7	0.36
C20 : 2	2.78	0.48	0.35	0.53	0.9	0.63	1.63
C20 : 3n6	1.75	4.33	3.79	2.75	1.95	0.85	1.07
C20 : 4n6	0.72	1.36	1.09	1.3	1.34	1.27	1.8
C20 : 3n3	0.08	—	—	—	7.07	88.67	6.29
C20 : 5n3	0.37	0.18	0.35	0.14	1.24	0.55	0.86
Sum	0.61	0.84	1.09	0.53	1.16	0.75	0.99

Note: * For fatty acid designations see Table 2.

Thus, our data and those from the literature allow us to propose some alternative pathways of arachidonic and eicosapentaenoic acid biosyntheses in different species of the genus *Porphyridium*.

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