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EFFECT OF SALINITY ON LIPID COMPOSITION OF *CLADOPHORA VAGABUNDA*

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Key Word Index—*Cladophora vagabunda*; Cladophoraceae; Chlorophyta; lipids; fatty acids; salinity.

Abstract—The main lipid classes and their fatty acid composition were investigated in *Cladophora vagabunda* inhabiting waters with different salinities or subjected to salt-stress. Most of the observed changes probably have adaptive value. Differences between adaptation and stress, and also the effects of the degree of the stress are discussed.

INTRODUCTION

Marine algae possess very complex and diverse lipid compositions. The concentration of polyunsaturated fatty acids (PUFA) in some is relatively high, and thus, is of practical interest for drugs and foods. In some cases, the lipid composition might also be useful as a chemotaxonomic marker [1]. There is limited information about the effect of environment on the lipid composition of algae. For marine algae, it could be expected that there would be some effect of salinity on the metabolism, especially on important cell membrane constituents such as lipids. In the unicellular marine red alga, *Porphyridium cruentum*, increased salinity causes a decrease of growth rate and concentrations of eicosapentenoic acid, while the concentrations of the arachidonic acid increase [2]. Total lipid content of the red microalga, *Dunaliella salina*, decreases at higher salinity [3]. The same was observed in the red algae, *Chondria tenuissima*, where, besides total lipids (TL), monogalactosyl diacylglycerols (MGDG) also decreased at higher salinities while unsaturated fatty acids (FA) concentrations increased [4]. Decreases of the concentrations of 16:0 and 16:1 FA and increases of those of 16:3 FA were observed when the microalga *Coscinodiscus eccentricus*, grows at high salinity [5]. However, the effect of salinity was insignificant in evolutionary higher green algae; in *Platymonas tetrathele* growing at different salinities, there were no differences in FA composition [6]. Contrary to these data, in *Ctenocladus circinnatus* a decrease of the growth rates was observed at increased salinity [7].

Evidently, there are many contradictory data about the effect of salinity on the metabolism of marine algae and only in some cases have lipid changes been investigated. For this reason, we performed an investigation on the lipid changes in *Cladophora vagabunda* collected from two locations with different salinities (Lake Pomorie, with 50‰ salinity, and a saltpan in the same region, with 200‰ salinity). The alga usually inhabits the Black Sea (salinity 16–18‰), but some of its spores penetrate into the lake and into the saltpan through channels and the growing plants must adapt to the higher salinity (see Fig. 1). This adaptation takes a long time and the changes obtained may be different from those caused by a rapid change of salinity. In order to compare the effects of slow adaptation and of a salt-stress we also performed some model experiments with the alga, placed suddenly in aquaria with large differences in salinity.

Representatives of the genus *Cladophora* (Chlorophyta, Siphonophyceae, Cladophoraceae) are distributed world-wide and often dominate the benthos in fresh and marine waters. They could be used as raw materials for industrial use. They also provide habitat and food for numerous organisms and their ability to survive up to 100‰ salinity makes them very important ecologically [8–10].

RESULTS

We compared separately the changes in lipid classes and FA composition found in the alga as a result of adaptation to different salinity and those due to salt-stress. The relative concentrations of the main lipid classes of *C. vagabunda* grown in waters with different

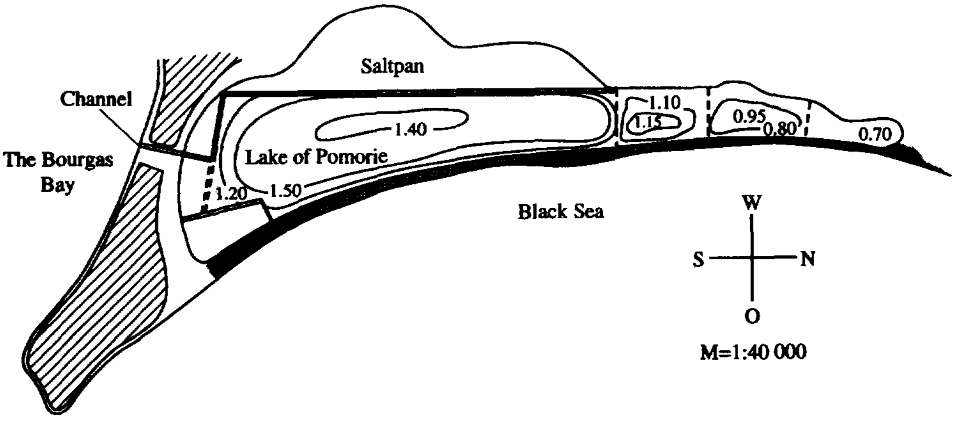

Fig. 1. Location of samples of *Cladophora vagabunda*.

Samples from the lake (salinity 50‰) and from the saltpan (200‰) showed significant differences in their lipid composition. Higher salinity lowered total lipid content twofold, which is in agreement with literature data [3, 4]. At lower salinity there was a high concentration of triacylglycerols (TAG), which comprised 86% of the total lipids, while in the sample from the saltpan they made up only 27%. In the latter, glyco-

Table 1. Amounts of main lipid classes in *Cladophora vagabunda* grown at different salinities or subjected to a salt-stress

Sample	Lipid class*	Lipid content mg g^{-1} dry wt*†	Lipid content % total
Lake (50‰ salinity)	TAG	2.73±0.27	86.1
	MGDG	0.13±0.01	4.1
	DGDG	0.11±0.01	3.5
	SQDG	0.13±0.01	4.1
	PL	0.07±0.01	2.2
	TL	3.17±0.31	100.0
Lake transferred to 200‰ salinity	TAG	2.11±0.21	78.4
	MGDG	0.14±0.02	5.2
	DGDG	0.25±0.03	9.3
	SQDG	0.13±0.01	4.8
	PL	0.06±0.01	2.2
	TL	2.69±0.28	99.9
Saltpan (200‰ salinity)	TAG	0.46±0.05	27.1
	MGDG	0.39±0.04	22.9
	DGDG	0.05±0.01	2.9
	SQDG	0.71±0.07	41.8
	PL	0.09±0.01	5.3
	TL	1.70±0.18	100.1
Saltpan transferred to 140‰ salinity	TAG	2.74±0.07	35.7
	MGDG	0.72±0.07	34.8
	DGDG	0.49±0.05	23.7
	SQDG	0.05±0.01	2.4
	PL	0.07±0.01	3.4
	TL	2.07±0.21	100.0
Saltpan transferred to 110‰ salinity	TAG	1.91±0.20	76.7
	MGDG	0.09±0.01	3.6
	DGDG	0.21±0.02	8.4
	SQDG	0.17±0.02	6.8
	PL	0.11±0.01	4.4

Table 2. Effect of salinity on fatty acid composition of main lipid classes in *Cladophora vagabunda*

	Lake (50‰ salinity)						Saltpan (200‰ salinity)					
tty acids	TL	TAG	MGDG	DGDG	SQDG	PL	TL	TAG	MGDG	DGDG	SQDG	PL
:0	7.1	6.4	6.6	7.4	18.1	16.6	7.0	8.2	4.1	8.6	7.5	9.0
:0	27.1	25.9	29.5	36.1	44.4	24.5	28.1	25.8	36.2	30.0	24.1	33.5
:1*	17.0	18.1	13.1	12.7	7.4	5.9	14.7	15.6	8.6	23.0	17.3	10.8
:2	0.9	0.9	–	2.5	–	–	2.3	1.8	2.9	9.9	1.7	0.9
:4	1.2	1.3	–	1.9	–	–	1.2	1.9	–	–	1.5	1.9
:0	2.1	2.0	2.4	3.5	–	4.9	3.9	3.1	6.6	3.7	3.0	3.2
:1*	8.5	8.5	6.9	6.3	12.1	7.1	25.2	25.5	27.3	16.9	24.8	25.9
:2*	6.3	6.2	7.2	9.6	5.8	5.4	10.3	9.2	12.0	7.8	10.2	11.4
:3	8.8	9.4	12.9	8.4	5.3	7.0	2.6	3.2	1.3	–	3.1	1.7
:4	2.8	2.9	6.0	1.3	–	1.9	0.6	0.5	–	–	1.2	–
:4 (*n*-6)	2.5	2.6	3.0	1.6	–	4.4	1.1	1.1	0.5	–	1.1	0.7
:5	7.8	8.4	7.5	3.2	–	7.0	2.4	3.2	0.6	–	3.2	1.0
:5	1.1	1.1	–	–	–	5.3	–	–	–	–	–	–
:6	1.0	1.1	–	–	–	1.7	0.6	1.1	–	–	1.4	–
tal	99.4	99.9	99.9	100.1	100	100.1	100	100	100	100	100.1	100
sat./sat.	1.8	2.9	1.6	1.1	0.6	1.2	1.6	1.7	1.1	1.4	1.9	1.2

*More than one isomer present.
See text for abbreviations.

Table 3. Effect of salt stress on fatty acid composition of main lipid classes of *Cladophora vagabunda*

	From 200‰ to 140‰ salinity						Sample transferred from saltpan from 200‰ to 110‰ salinity						Sample transferred from lake from 50‰ to 200‰ salinity					
y acids	TL	TAG	MGDG	DGDG	SQDG	PL	TL	TAG	MGDG	DGDG	SQDG	PL	TL	TAG	MGDG	DGDG	SQDG	PL
)	10.6	9.2	6.7	17.6	15.2	12.2	8.0	6.5	20.6	10.3	9.2	16.7	7.3	6.2	11.3	11.4	12.8	10.2
2	32.2	27.7	35.2	32.8	44.9	34.9	21.8	20.8	19.7	17.0	38.3	25.8	27.2	25.9	35.3	25.8	42.2	26.6
1*	11.9	14.5	9.8	11.6	13.2	8.5	19.1	20.7	7.8	17.6	8.0	21.0	21.2	24.1	10.4	13.5	7.5	8.7
2	3.5	2.7	2.0	6.6	6.4	0.9	1.5	1.4	–	3.1	–	3.2	1.2	1.2	1.6	2.1	0.3	–
4	–	0.9	–		–	–	1.3	1.3		3.9	–	–	1.5	1.5	1.6	2.5	0.8	–
)	3.9	3.2	4.9	3.5	2.6	4.7	1.6	1.7	3.0	–	2.5	–	1.1	1.0	1.3	1.2	1.5	4.3
1*	23.0	23.9	26.9	16.3	12.2	25.6	15.8	15.2	20.1	11.0	21.8	5.9	7.0	6.3	10.3	7.6	14.0	8.5
2*	9.3	10.6	11.1	6.9	5.4	11.0	7.1	7.0	6.1	11.3	5.3	10.7	6.4	7.5	8.2	18.5	4.7	–
3	1.0	1.9	1.0	–	–	1.1	7.7	8.0	5.5	10.6	6.0	4.3	8.0	8.3	4.8	9.7	4.4	7.1
4	0.5	0.9	0.4	–	–	–	2.3	2.7	1.7	1.8	–	2.2	2.8	3.4	1.3	0.7	0.3	1.2
4 (*n*-6)	0.6	1.0	0.6	–	–	–	3.0	3.0	3.9	2.4	2.6	–	2.0	2.0	2.5	1.7	0.7	3.5
5	2.6	2.6	1.4	4.9	–	1.1	9.0	9.7	6.0	9.3	3.6	3.8	7.1	8.1	5.0	3.3	1.1	5.1
5	–	–	–	–	–	–	1.3	1.0	5.8	1.7	2.7	6.5	1.0	1.1	1.1	–	–	1.7
)	0.6	0.9	–	–	–	–	0.8	1.1	–	–	–	–	0.8	0.9	–	–	0.6	1.9
1	99.7	100	100	100.2	99.9	100	100	100.1	100.2	100	100	100	99.4	99.6	99.6	99.8	100.1	100.1
ıt./sat.	1.1	1.5	1.1	0.9	0.6	0.9	2.2	2.5	1.3	2.7	1.1	1.4	1.8	2.2	1.1	1.6	0.8	1.4

ɅIore than one isomer present.
:e text for abbreviations.

lipids (GL) predominated, the main class being sulphoquinovosyl diacylglycerols (SQDG). Extremely low amounts of digalactosyl diacylglycerols (DGDG) were found in this sample. In both cases, phospholipids were present in low concentrations.

The FA profiles (Table 2) of the samples from the saltpan and the Lake of Pomorie were similar. The main constituent appeared to be 16:0, which is typical of *C. vagabunda* [11–13], as well as for other marine [1, 14–16] and freshwater [17] *Cladophora* species. The amounts of 14:0 and 16:1 acids were also relatively high, as was observed in other *C. vagabunda* samples [11, 12], but it must be noted that a sample of the same alga from the Black Sea contains only very small amounts of these FA [13]. Nevertheless, the total FA in both samples showed some quantitative differences. In the sample from the lake with lower salinity all C_{18} acids, with the exception of linolenic acid, were found in lower concentrations, while the concentrations of PUFA were higher; 20:5 predominated in this group.

There were larger differences in the FA composition of the separated lipid classes. In the sample from the lake (lower salinity), 16:0 occurred in higher concentrations in SQDG, while in the sample from the saltpan it was higher in MGDG and DGDG. In the total FA from both samples, differences in the unsaturated/saturated acid ratio were very small but there were significant ones in different lipid classes. In the sample of the alga inhabiting water with lower salinity, the most unsaturated lipid class was TAG, while the most saturated was SQDG. On the other hand, the most unsaturated classes in the saltpan sample were SQDG and TAG, while MGDG were the most saturated.

We also found that there were different changes in the lipid composition caused by salt stress (model experiments with radical increases or decreases of salinity). The changes in the lipid composition of *C. vagabunda*, transferred from the lake to the saltpan were relatively small, although this was the biggest salt-stress in all our experiments—a salinity change from 50‰ to 200‰. The amount of the total lipids decreased after the transfer to the saltpan, analogous to adaptive changes [3, 4]. The amount of total GL increased, but this was due almost entirely to DGDG. The increase of the total GL was in agreement with the above-mentioned observation that in the sample from the higher salinity the concentration of the GL was much higher. As found in both control samples, PL did not change their concentrations after the change in salinity.

After transfer of the alga to the saltpan, there were almost no changes in the concentrations of different FA in total lipids but there were some in the lipid classes, mainly associated with unsaturation. In TAG and MGDG it decreased after transfer to the saltpan, while in DGDG and PL it increased.

decrease of the salinity, which is in agreement with previous data [3, 4]. TAG concentrations also increased proportionally with the decrease of salinity and at 110‰ salinity approached those in the lake samples. Changes in GL were more significant than in PL concentrations, but the changes in the sample from 140‰ salinity were more significant than those at 110‰. At 140‰ the concentrations of MGDG and DGDG were higher and those of SQDG and PL lower. At 110‰ the above-mentioned concentrations were closer to those found in the samples from the lake.

Similar to the lipid classes, the changes in the composition of most of the FA were more substantial in the samples cultivated at 140‰ salinity, but some of the FA changed their concentrations proportionally with the salinity. At the lowest salinity (110‰), concentrations of FA were closer to those in the sample from the lake. For example, the concentration of 18:1 acid decreased with salinity and appeared closer to that in the lake sample. PUFA concentrations increased with the decrease in salinity and approached closely those from the alga inhabiting the lake. In general, unsaturation of FA showed a minimum at 140‰ and maximum at 110‰, which was in agreement with higher unsaturation in the sample from the lake (lower salinity).

In the different lipid classes, there were also significant FA changes after incubation at lower salinity. 16:0 concentrations in SQDG showed significant increases at lower salinity, which were also characteristic of the sample from the lake. In the sample from the saltpan, PUFA, were concentrated mainly in TAG and SQDG, but at reduced salinity, in TAG and DGDG. In all samples from the basin with lower salinity, including that from the lake, unsaturation in SQDG appeared to be the lowest.

DISCUSSION

There are differences between the lipid composition of *C. vagabunda*, living at different salinities. The alga normally inhabits the Black Sea (16–18‰) but both of the samples investigated by us are from plants growing at higher salinities (lake, 50‰; saltpan, 200‰). Probably, the increased salinity is the reason for the relatively lower concentrations of MGDG in both samples, compared with those in the Black Sea [13]. It is known that, because of its hexagonal (2)-structure, a reduced level of MGDG may indicate a higher degree of control of ionic permeability of cell membranes [18] and, thus, may have an adaptive value towards higher salinity. The increased concentrations of saturated FA in the alga from the saltpan also could be an adaptive mechanism, because they lower the cell membrane permeability.

In model experiments, incubating *C. vagabunda* from the lake and from the saltpan at different salinities resulted in salt stress, owing to the radical change of were also some changes, common to all experiments. Total lipid concentrations in all algal samples increased proportionally to the decrease of the salinity. This was observed in other algae and our experiments confirm the relationship between total lipid content and salinity. We found analogous changes in TAG, with their concentrations increasing proportionally with the decrease of salinity. There were also significant changes in their FA composition. These results were an indication that TAG may have a more important role in the adaptation and survival of algae in waters with different salinities than just an energy resource only. One possible explanation is our recent observation that in some Black Sea algae, including *Cladophora* sp., the surface exudates contain mainly TAG (more than 60% of total exudates) and this is an indication of the protective role of TAG in algae.

There were very low concentrations of phospholipids in all samples investigated and the changes in the concentrations of these lipid classes at different conditions, and those in FA, were relatively small. These data indicate that phospholipids are not affected by the salt-stress.

Compared with PL, the changes in total GL and in different GL groups were relatively large. These compounds are of special importance for chloroplasts and their changes probably have some adaptive value, through photosynthetic processes.

It must be mentioned, that changes observed after the model experiments showed a general tendency to create a lipid composition similar to that for *C. vagabunda* living in waters with similar salinity. This means that even with drastic salinity changes, which we can call 'salt stress', for only 75 hr there are significant lipid changes with adaptive value.

While in the model experiments the concentrations of the total lipids and TAG changed proportionally with the salinity, those of the polar lipids (GL and PL) showed unexpected maxima or minima at an average salinity (140‰). Analogous changes in the lipid and sterol composition we observed earlier in lipids of some desiccation-tolerant plants [19], where at 50% water-deficit there were more significant changes (maxima or minima of the corresponding concentrations) than in fully dried plants (water-deficit 87%). This shows that in different plants adaptive changes of cell membrane constituents (polar lipids and sterols) are not always proportional to the degree of environmental changes and probably very great stress can inhibit adaptive reactions.

EXPERIMENTAL

Plant material and analysis. Fresh algae were collected in Lake Pomorie and in a saltpan near Pomorie in the Bourgas Bay, southern Bulgarian Black Sea coast, in September 1993. Voucher specimens are

Model experiments on decreased water salinity. Samples (*ca* 80 g fresh wt) from the saltpan were transported in H_2O and placed in two 70-l aquaria; H_2O salinity was adjusted to 140‰ and 110‰ with sea H_2O (salinity 18‰). After 75 hr incubation with constant aeration, plants were dipped in EtOH and investigated as described above. The temp. was maintained between 23 and 25° in a 12.5/11.5 hr light/dark period.

Model experiments on increased water salinity. A sample from Lake Pomorie was transported in H_2O to the saltpan and placed within a plastic net for 75 hr. The analyses were then performed as described above.

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