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Fatty acid composition of individual polar lipid classes from marine macrophytes

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Abstract

Major glycolipids [monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG)) and phospholipids (phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG)] as well as betaine lipid 1,2-diacylglycero-*O*-4′-(*N,N,N*-tri-methyl)-homoserine (DGTS) were isolated from *Anfeltia tobuchiensis* (Rhodophyta), *Laminaria japonica*, *Sargassum pallidum* (Phaeophyta), *Ulva fenestrata* (Chlorophyta) and *Zostera marina* (Embriophyta), harvested in the Sea of Japan. GC analysis of their fatty acid (FA) composition revealed that the n-6 polyunsaturated FAs (PUFAs) shared the most part of the sum of n-6 and n-3 PUFAs in PC and PE compared with glycolipids and PG. In algae, it was related to the prevalence of 20:4n-6 over 20:5n-3 in non-photosynthetic lipids. Percentage of n-6 PUFAs as well as the sum of n-3 and n-6 PUFAs decreased in the following sequence: PC→PE→PG. The saturation increased in the lines of MGDG→DGDG→SQDG and PC→PE→PG. PG was close to SQDG by the level of saturation. Distribution of C_{18} and C_{20} PUFAs in polar lipids depended on taxonomic position of macrophytes. Balance between C_{18} and C_{20} PUFAs was preferably shifted to the side of C_{20} PUFAs in PC and PE that was observed in contrast to glycolipids and PG from *L. japonica* containing both series of FAs. The set of major FAs of polar lipid classes can essentially differ from each other and from total lipids of macrophytes. For example, MGDG was found to accumulate characteristic fatty acids 16:4n-3, 16:3n-3, 18:3n-6 and 18:4n-3, 20:3n-6 in *U. fenestrata, Z. marina, L. japonica* and *S. pallidum,* respectively.

Keywords: *Anfeltia tobuchiensis*; *Laminaria japonica*; *Sargassum pallidum*; *Ulva fenestrata*; *Zostera marina*; Algae; Seagrass; Fatty acids; Glycolipids; Phospholipids; Betaine lipid DGTS

1. Introduction

Marine macrophytes are a phylogenetically diverse group of plants. They comprise evolutionary far lines (South and Whittick, 1987) such as macroalgae, belonging to three main taxonomic groups (Chlorophyta, Phaeophyta and Rhodophyta), and seagrasses (Embryophyta). The interest, attracted to marine macrophytes, is caused by various kinds of their biological activity. They possess antimicrobial, antiviral, anti-inflammatory and immunotropic properties. These features may be primarily related to the high content of different glycolipids [monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG)], which along with phospholipids [phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG)] are the main polar lipids of marine macrophytes (Kayama et al., 1989; Dembitsky et al., 1991; Thompson, 1996). There is some current data available suggesting the biological activity of glycolipids. MGDG from green algae is shown to reveal anti-tumor-promoting effect (Morimoto et al., 1995). SQDG from algae inhibits DNA-polimerase and HIV-reverse transcriptase (Gustafson et al., 1998; Loya et al., 1998; Ohta et al., 1998). It is well known that biological activity of marine macrophytes is related to the essential polyunsaturated fatty acids (PUFAs), which are the abundant components of macrophytic glycolipids (Khotimchenko, 1993a, b; Goncharova et al., 2000; Sanina et al., 2000). Marine macrophytes are poikilothermic organisms. Fatty acid constituents of lipids (Jones and Harwood, 1975; Sanina et al., 1987; Melo et

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al., 1995; Kim et al., 1996) and, therefore, membrane fluidity of poikilotherms (Williams et al., 1996; Sewón et al., 1997; Sanina et al., 2003) are very sensitive to the environmental temperature. However, screening of marine macrophytes revealed that composition of their polar lipids and total fatty acids (FAs) primarily reflects the taxonomic position of these plants (Khotimchenko, 1999). Despite of the wide range of data on fatty acid composition of total lipids, it is a little known about fatty acid composition of individual lipid classes of marine macrophytes (Smith and Harwood, 1984; Arao and Yamada, 1989). Earlier, we have characterized fatty acid composition of phospho- and glycolipids, isolated from two macrophytic species (brown alga *L. japonica* and seagrass *Z. marina*), for the interpretation of thermotropic behavior of these lipids (Goncharova et al., 2000; Sanina et al., 2000, 2003). Present paper represents data obtained by investigation of 5 species from all four divisions of marine macrophytes. Fatty acid composition of lipids from *A. tobuchiensis* has not been characterized before in spite of commercial importance of this red alga as a raw material for the agar production. The purposes of our work were to clarify which fatty acids could mainly define thermotropic behavior of the major glyco-, phospho- and betaine lipid classes, isolated from different species of marine macrophytes, and to compare the acyl residue composition of two lipid groups. First group included MGDG, DGDG, SQDG and PG, and the second one comprised PC and PE. As known, these groups of polar lipids mainly occur in photosynthetic and non-photosynthetic membranes, respectively (Harwood, 1998).

2. Results and discussion

Most of marine macrophytes contain major membrane lipids as higher plants. There are glycolipids (MGDG, DGDG and SQDG) and phospholipids (PC, PE and PG). Unusual compounds such as betaine lipids can be also present as the major constituents in many species of algae (Eichenberger and Araki, 1993; Harwood, 1998). Essential content of betaine lipid DGTS was found in green algae, where it seems to substitute PC (Thompson, 1996). The results of GC analysis of the acyl chain distribution among these polar lipids isolated from 5 species of marine macrophytes: *A. tobuchiensis* (Rhodophyta), *L. japonica* and *S. pallidum* (Pheaophyta), *U. fenistrata* (Chlorophyta) and Z. *marina* (Embryophyta) are summarized in Tables 1–5.

2.1. Phospholipids

Phospholipids were isolated from 3 species of marine macrophytes: red algae *A. tobuchiensis,* brown algae *L. japonica* and seagrasses *Z. marina*. They contain the highest percentage of phospholipids (30.6–51.4% from the total lipids) in comparison with other species, *S. pallidum* and *U. fenistrata* (12.6% and 17.2% from the total lipids, respectively) (Goncharova, 2003). Three major plant phospholipids (PC, PE and PG) are differed by their relative content (Table 6), location in cells, participation in photosynthetic machinery and biosynthetic pathways. PC and PE are the main components of extra-plastidial membranes. They are synthesized at the endoplasmic reticulum, whereas biosynthesis and localization of PG are mainly associated with chloroplasts (Thompson, 1996). In higher plants, PG is not exclusively found in chloroplast, but in extra-plastidial membranes also (Joyard et al., 1998), that is presumably true for marine macrophytes.

As shown in Table 1, there are six major FAs, 16:0, 18:0, 18:1n-9, 18:1n-7, 20:4n-6 and 20:5n-3, in phospholipids of *A. tobuchiensis*. Additionally, PC and especially PE contain essential amounts of dihomo-γ-linolenic acid 20:3n-6, which was not detected in PG. This FA was found as a minor component of total lipids in other red algae. It is considered as an intermediate compound in biosynthesis of arachidonic acid (Khotimchenko, 1999). 16:0, 18:1n-9, 20:4n-6 and 20:5n-3 are

Table 1
Fatty acid composition of major polar lipids of red algae *Anfeltia tobuchiensis* (% of the sum of fatty acids)

Fatty acids	Lipid classes					
	PC	PE	PG	MGDG	DGDG	SQDG
14:0	0.6	0.7	0.2	0.4	0.6	4.2
15:0	0.3	0.6	0.2	n.d.	0.2	1.5
16:0	12.5	15.4	41.2	10.5	31.5	32.3
16:1	0.9	2.7	0.6	1.4	0.9	3.4
16:2	n.d.	0.5	1.5	n.d.	0.1	n.d.
17:0	0.7	0.5	0.3	1.0	0.3	0.5
18:0	4.1	6.0	4.8	0.9	4.1	8.7
18:1n-9	21.6	21.0	20.7	8.1	25.4	10.0
18:1n-7	14.3	6.6	4.7	1.5	n.d.	2.5
18:2n-6	1.1	1.1	0.7	0.6	1.0	1.2
20:2n-6	0.8	1.9	n.d.	n.d.	n.d.	n.d.
20:3n-6	2.7	9.9	n.d.	0.6	1.8	n.d.
20:4n-6	28.3	20.6	14.5	36.8	19.7	20.1
20:5n-3	6.7	6.7	8.5	36.8	13.2	13.0
SFA	18.2	25.0	47.8	13	37	48
MUFA	40	33	27	11	27	16
PUFA	41.8	42	25.2	76	36	35
UI	199	188	132	347	180	164
Saturated/unsaturated	0.22	0.33	0.92	0.15	0.59	0.96
C_{18} PUFA	1.1	1.1	0.7	0.6	1.0	1.2
C_{20} PUFA	38.5	39.1	23.0	74.2	34.7	33.1
n-3	6.7	6.7	8.5	36.8	13.2	13.0
n-6	32.9	33.5	15.2	38.0	22.5	21.3
Σ (n-3 + n-6)	39.6	40.2	23.7	74.8	35.7	34.3

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.

n.d., not detected.

Table 2
Fatty acid composition of major polar lipids of brown algae *Laminaria japonica* (% of the sum of fatty acids)

Fatty acids	Lipid classes						% FA from total lipids[a]
	PC	PE	PG	MGDG	DGDG	SQDG	
14:0	12.7	4.4	0.8	5.0	9.0	3.6	5.3
16:0	12.4	29.3	14.4	5.5	20.0	45.2	12.3
16:1	4.4	6.1	1.7	4.0	20.5	4.3	3.9
18:0	0.7	3.8	23.0	0.5	3.3	3.6	1.0
18:1n-9	12.1	9.2	15.7	9.6	14.1	21.9	8.4
18:2n-6	12.4	5.4	13.7	11.1	10.9	7.7	8.4
18:3n-6	0.4	0.7	1.2	8.0	1.8	0.9	4.2
18:3n-3	1.3	2.8	8.7	8.7	5.2	3.0	6.1
18:4n-3	0.4	0.8	0.3	20.3	3.2	0.9	13.9
20:4n-6	29.1	26.9	4.5	9.9	2.1	3.0	14.0
20:5n-3	7.5	2.3	0.3	15.9	3.4	1.4	14.0
SFA	27.6	40.0	46.9	11.5	35.0	53.1	
MUFA	17.6	19.9	20.6	14.0	37.7	30.0	
PUFA	54.8	40.1	32.5	74.5	27.3	16.9	
UI	214	169	110	387	120	79	
Saturated/unsaturated	0.38	0.66	0.88	0.13	0.54	1.13	
C_{18} PUFA	14.5	9.7	23.9	48.1	21.1	12.5	32.8
C_{20} PUFA	36.6	29.2	4.8	25.8	5.5	4.4	29.3
n-3 PUFA	9.2	5.9	9.3	44.9	11.8	5.3	34.7
n-6 PUFA	41.9	33	19.4	29	14.8	11.6	28.4
Σ (n-3 + n-6)	51.1	38.9	28.7	73.9	26.6	16.9	63.1

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.

n.d., not detected.

[a] Khotimchenko (1998).

Table 3
Fatty acid composition of major polar lipids of seagrasses *Zostera marina* (% of the sum of fatty acids)

Fatty acids	Lipid classes				% FA from total lipids[a]
	PC	PE	MGDG	DGDG	
16:0	20.4	33.1	5.8	10.8	16.8
16:1	0.5	0.3	1.6	1.1	2.4
16:2	0.1	0.2	2.5	1.4	0.8
16:3n-3	0.2	n.d.	15.0	1.8	8.6
18:0	1.6	0.8	1.1	1.3	1.1
18:1n-9	1.6	0.9	3.1	1.9	1.7
18:2n-6	37.4	32.8	10.0	10.3	15.7
18:3n-3	35.6	30.8	58.8	70.0	48.6
20:5n-3	1.9	n.d.	1.2	1.4	0.2
SFA	22.5	34.9	7.7	12.1	
MUFA	2.3	1.3	4.7	3.0	
PUFA	75.2	63.8	87.6	84.9	
UI	194	160	258	249	
Saturated/unsaturated	0.29	0.54	0.08	0.14	
C_{18} PUFA	73.0	63.6	68.8	80.3	
C_{20} PUFA	1.9	n.d.	1.2	1.4	
n-3	37.7	30.8	75	73.2	
n-6	37.4	32.8	10.0	10.3	
Σ (n-3 + n-6)	75.1	63.6	85.0	83.5	

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.

n.d., not detected.

[a] Khotimchenko (1993b).

Table 4
Fatty acid composition of major polar lipids of brown algae *Sargassum pallidum* (% of the sum of fatty acids)

Fatty acids	Lipid classes			% FA from total lipids[a]
	MGDG	DGDG	SQDG	
14:0	3.7	2.7	3.2	3.6
16:0	13.2	12.5	16.6	22.4
16:1	3.2	4.7	4.9	6.3
17:0	1.4	5.0	13.4	<1
16:4n-3	0.3	0.4	8.8	n.d.
18:0	1.6	1.7	5.4	0.8
18:1n-9	6.0	3.5	7.0	7.2
18:2n-6	16.3	7.0	2.9	9.8
18:3n-3	5.1	11.2	5.1	7.2
18:3n-6	1.6	1.0	n.d.	1.3
18:4n-3	7.1	14.6	5.3	7.3
20:1n-9	0.9	0.3	1.3	0.9
20:2n-6	0.4	0.1	1.4	0.3
20:3n-6	6.0	0.9	1.3	3.6
20:4n-3	0.8	0.4	1.2	0.3
20:4n-6	15.6	10.0	1.0	18.0
20:5n-3	12.6	14.2	0.4	3.8
22:0	0.4	0.3	1.5	n.d.
22:2n-6	0.3	0.1	1.4	n.d.
22:3n-6	n.d.	0.2	2.7	n.d.
SFA	21.0	24.1	50.5	
MUFA	11.3	13.5	17.4	
PUFA	67.7	62.4	32.1	
UI	248	247	125	
Saturated/unsaturated	0.27	0.32	1.02	
C_{18} PUFA	30.1	33.8	13.3	
C_{20} PUFA	35.4	25.6	5.3	
n-3	25.9	40.8	20.8	
n-6	40.2	19.3	10.7	
Σ (n-3 + n-6)	66.1	60.1	31.5	

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.

n.d., not detected.

[a] Khotimchenko (1991).

Table 5
Fatty acid composition of major polar lipids of green algae *Ulva fenestrata* (% of the sum of fatty acids)

Fatty acids	Lipid classes				% FA from total lipids[a]
	MGDG	DGDG	SQDG	DGTS	
16:0	1.1	24.8	58.7	35.5	29.9
16:1	0.3	0.9	1.5	1.8	1.9
16:1n-6	0.3	2.5	n.d.	0.2	n.d.
16:3n-3	1.0	3.6	n.d.	0.6	1.7
16:4n-3	43.7	2.6	n.d.	0.3	9.3
18:0	0.2	0.9	1.6	0.4	1.0
18:1n-7	1.8	5.0	15.4	8.4	8.4
18:1n-9	0.4	1.6	0.2	1.2	2.5
18:2n-6	2.1	14.2	2.1	6.9	9.8
18:3n-3	24.1	13.8	18.6	14.6	15.4
18:3n-6	0.3	n.d.	n.d.	5.5	1.1
18:4n-3	24.3	23.8	1.6	15.8	6.2
20:4n-6	n.d.	n.d.	n.d.	4.9	1.8
20:5n-3	tr.	n.d.	n.d.	3.5	1.7
SFA	1.3	26.0	60.6	36.3	
MUFA	2.5	7.5	17.1	11.6	
PUFA	96.2	66.5	22.3	52.1	
UI	357	217	84	189	
Saturated/unsaturated	0.01	0.35	1.54	0.57	
C_{18} PUFA	50.8	51.8	22.3	42.8	
C_{20} PUFA	tr.	n.d.	n.d.	8.4	
n-3 PUFA	93.1	43.8	20.2	34.8	
n-6 PUFA	2.4	14.2	2.1	17.3	
Σ (n-3 + n-6)	95.5	58.0	22.3	52.1	

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.

n.d., not detected; tr., traces (content less than 0.1%).

[a] Khotimchemko (1993a).

the major FAs in contrast with others (18:0 and 18:1n-7) in total lipids of other red algae (Dembitsky et al., 1991; Khotimchenko and Vaskovsky, 1990; Vaskovsky et al., 1996). Uncommon *cis*-vaccenic acid 18:1n-7 was especially concentrated in PC (14.3% against ca. 5–6% in PE and PG), whereas it accounts only ca 1–2% in total lipids of Rhodophyta. Percentage of its positional isomer 18:1n-9 also reached much higher level (at least 2 fold) in all phospholipid classes of *A. tobuchiensis* in comparison with total lipids in Rhodophyta. Percentage of 14:0 did not exceed 1% in phospholipid classes of *A. tobuchiensis*, whereas it was one of the major fatty acids in total lipids of other species of Rhodophyta.

One more interesting feature of fatty acid composition of phospholipids concerns C_{20} PUFAs 20:4n-6 and 20:5n-3. Biological functions of these long-chain PUFAs in marine algae are unknown. An abundance of arachidonic and eicosapentaenoic acids in red and brown algae allows suggesting that they can play the role of eicosanoid's precursors as it was found in animals. It seems to be possible, since very low percentage of linoleic acid and α-linolenic acids was observed in *A. tobuchiensis*. Indeed it is intriguing that eicosanoids resemble the closely related lipoxigenase-derived metabolites of abundant linoleic and α-linolenic acids in higher plants (Marks, 1999). The content of 20:4n-6 was much higher and more variable in comparison with 20:5n-3 in phospholipids of *A. tobuchiensis*. The domination of arachidonic acid over eicosapentaenoic acid was more expressed in PC and PE than in PG (ca. 4- and 3-fold against less than 2-fold, respectively). The opposite ratio between 20:4n-6 and 20:5n-3 was found in total lipids of majority of other red algae (Khotimchenko and Vaskovsky, 1990).

As shown earlier, the ratio between 20:4n-6 and 20:5n-3 of PC and PE from brown algae *L. japonica* was higher in summer than in winter (Sanina et al., 2003). Increasing ratio of 20:4n-6/n-3 PUFAs, in PC and PE was suggested to be related to the requirement of much higher potent "n-6 eicosanoids" then those derived from

Table 6
Relative amounts of the major polar lipids from marine macrophytes (Goncharova, 2003)

Species	PC	PE	PG	MGDG	DGDG	SQDG
	% of total phospholipids			% of total glycolipids		
Anfeltia tobuchiensis	70.9	6.6	18.1	26.0	64.0	10.0
Laminaria japonica	28.4	16.5	25.8	47.0	38.0	15.0
Sargassum pallidum	—	52.1	28.1	70.7	22.2	7.1
Ulva fenestrata	—	16.3	35.6	70.8	19.5	9.7
Zostera marina	38.2	17.7	23.2	35.0	58.0	6.2

n-3 PUFAs (Lauritzen et al., 2001) in the most active seasonal period of other marine poikilothermic organisms, invertebrates (Sanina and Kostetsky, 2002). In marine macrophytes, environmental impact can induce signaling processes both in plasma membranes, mainly composed from PC and PE, and in chloroplast membranes. The main initiating process arises in the former (Tarchevsky, 2002) and therefore could demand more powerful mediators participating in the signal transduction.

Acyl composition of PC and PE, characterizing non-photosynthetic membranes, was more similar between each other than with "photosynthetic" PG. In particular, the content of 16:0 was ca. 3 times higher in PG in comparison with PC and PE. On the contrary, percentage of 20:4n-6 was ca. 2 and 1.5 times higher in PC and PE in comparison with PG, respectively. 20:3n-6 was completely absent in PG. n-3 and n-6 PUFAs of polar lipids from *A. tobuchiensis* mostly belonged to C_{20} series. The sum of PUFAs was the highest in PC and PE of *A. tobuchiensis*, where n-6 PUFAs substantially prevailed. In result, PG was the most saturated major phospholipid of *A. tobuchiensis*.

The majority of FAs, characterizing total lipids of *L. japonica* (Khotimchenko, 1998) dominated in phospholipid classes of this brown alga (Table 2). The list of their major FAs was more mosaic in comparison with phospholipids of red alga *A. tobuchiensis*. It comprised satd FAs, 14:0 and 16:0, monoene FAs, 16:1 and 18:1n-9, and PUFAs, 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3. Moreover, significant amount of 18:0 was identified in PG (23.0%), while PE and especially PC comprised much lower amount of this satd acid (3.8 and 0.7%, respectively) that was rather close to its amount of total lipids (Khotimchenko, 1998).

On the other hand, PG was characterized by relatively low amounts of 14:0, 16:1, 20:4n-6 and 20:5n-3 in contrast to PC and PE. On the contrary, percentage of 18:3n-3 was significant in PG (8.7%) in comparison with its small amount in PC and PE (1.3 and 2.8%, respectively). The revealed dependence didn't occur in such abundant FAs as 16:0 and 18:2n-6. The content of 16:0 was 2-fold higher, in turns, the percentage of 18:2n-6 was ca. 2 fold lower in PE compared to PC and PG. The main differences in fatty acid composition of total lipids were observed in the content of PUFAs. As earlier shown, dominant C_{18} and C_{20} PUFAs, 18:4n-3, 20:4n-6 and 20:5n-3, were present by ca equal amounts in total lipids of *L. japonica* (Khotimchenko, 1998). But we have found that the amount of 18:3n-6 and 18:4n-3 did not exceed more than 1% in phospholipids. Percentage of α-linolenic acid 18:3n-3 in PG only was comparable with one in total lipids of *L. japonica* (8.7 and 6.1%, respectively). Two major C_{20} PUFAs of total lipids of *L. japonica,* 20:4n-6 and 20:5n-3, were also accumulated in different phospholipids. Arachidonic acid dominated in two major phospholipids of non-photosynthetic membranes, PC and PE (29.1 and 26.9%, respectively). It was ca. 2 fold higher in comparison with total lipids. Similarly with *A. tobuchiensis*, the content of 20:4n-6 substantially prevailed the percentage of 20:5n-3 in PC and PE (ca. 4 and 11 times, respectively), whereas total lipids contained ca equal amounts of these C_{20} PUFAs. The predominance of 20:4n-6 over 20:5n-3 was apparent also in "photosynthetic" PG despite of much less content of both C_{20} PUFAs. In result, the sum of n-6 PUFAs was ca. 2–5 fold larger than the sum of n-3 PUFAs in all phospholipids. The similar peculiarity occurred in phospholipids of red alga *A. tobuchiensis*.

According to the values both of ratio SFA/UFA and UI, PG was the most satd phospholipid. In part PE was more satd lipid than PC. It was also observed in *A. tobuchiensis*. The sum of PUFAs was maximal in PC, while it was minimal in PG. The domination of n-6 PUFAs over n-3 PUFAs, more expressed in PC and PE compared to PG and glycolipids, was also the characteristic trait of polar lipids from *L. japonica*. Despite of the much higher content of C_{18} PUFAs in polar lipids of *L. japonica* compared to *A. tobuchiensis*, the prevalence of C_{20} PUFAs over C_{18} PUFAs occurred in phospholipids of both algal species.

The list of major fatty acids of PC and PE from *Z. marina* comprised only three major FAs, 16:0, 18:2n-6 and 18:3n-3, the content of which varied in the range of 20.4–37.4% (Table 3). Thus, the number of major fatty acids of phospholipids studied was some shorter than ones of total lipids from seagrass. The deal with that the content of 16:3n-3 was very low or not detected in phospholipids, while this PUFA amounted 8.6% in total lipid extract of *Z. marina* (Khotimchenko, 1993b). In turn, phospholipids were ca. 1.5–2 fold more abundant in 16:0 and 18:2n-6 compared to total lipids. Similar to other higher plants and in contrast to red algae *A. tobuchiensis*, C_{18} PUFAs almost completely substitute C_{20} PUFAs in major polar lipids and in total lipids of *Z. marina*. The sum of n-3 and n-6 PUFAs in PC and PE was the highest compared to two algal species (75.1 and 63.6% against 38.9–51.1%, respectively). At that, PC and PE were the most abundant in n-6 PUFAs compared to glycolipids (37.4

and 32.8% against ca. 10%, respectively). While amount of n-3 PUFAs was as high as percentage of n-6 PUFAs in phospholipids of seagrass, the firsts of them were much more representative, ca. 8 times prevailing n-6 PUFAs in glycolipids. Then, it may be suggested the greatest importance of n-3 PUFAs contained in lipids of photosynthetic membranes, whereas n-6 PUFAs are necessary first of all in polar lipids of extra-plastidial membranes. As a whole, PC was more unsaturated lipid than PE similar with two macrophytic algae *A. tobuchiensis* and *L. japonica* in spite of their substantial chemotaxonomic differences.

2.2. Glycoglycerolipids

Fatty acid composition of glycolipid classes, analyzed in all five species of studied marine macrophytes, is shown in Tables 1–5. The ratio between glycolipids is different in studied macrophytic species (Table 6). MGDG and DGDG were the dominant glycolipid classes, though the percentage of MGDG is not always higher than one of DGDG. In particular, DGDG prevailed over other glycolipids in *A. tobuchiensis* and *Z. marina* (Goncharova, 2003).

The set of major FAs of glycolipids from *A. tobuchiensis* comprises two acids less in comparison with phospholipids due with low amounts or the absence of 18:1n-7 and 20:3n-6. It should be point out that the later also was absent in PG. As a result, glycolipids of *A. tobuchiensis* and total lipids of other red algae (Khotimchenko and Vaskovsky, 1990) are characterized by more similar pools of major fatty acids in comparison with phospholipids. There were 16:0, 18:0, 18:1n-9, 20:4n-6 and 20:5n-3 among major FAs of glycolipids from *A. tobuchiensis*, though the content of stearic acid in MGDG was less than 1%. The appreciable amount of 14:0 (4.2%), which is one of the major FAs of total lipids isolated from Rhodophyta, was revealed in SQDG only, whereas it was less than 1% in other glycolipids and phospholipids. The highest percentages of 16:0, one of dominant FAs, were detected in DGDG and SQDG (little above 30%). It was ca. 3 times larger in comparison with MGDG. As mentioned, the distinguished trait of PG was also the especially high level of this satd FA (41.2%). Glycolipids of *A. tobuchiensis* contained one major monoene acid, 18:1n-9, percentage of which was ca. 2.5 times higher in DGDG than it was observed in MGDG and SQDG. In contrast with phospholipids, especially with PC and PE, glycolipids contented only low amount of *cis*-vaccenic acid. In general, the content both of SFA and MUFA was the lowest in MGDG in comparison with other polar lipids of *A. tobuchiensis*. On the contrary, PUFAs, almost completely belonging to C_{20} series, were mainly concentrated in MGDG (74.2% compared to 23.0–39.1% in other polar lipids). First of all, it was occurred at the expense of the increased content of eicosapentaenoic acid in this galactolipid. The content of 20:5n-3 was also remarkably higher in two other glycolipids in comparison with phospholipids. Therefore ratio between major PUFAs, 20:4n-6 and 20:5n-3, as well as between n-6 and n-3 PUFAs was much less in glycolipids compared to PC and PE (1.0–1.8 times against ca. 5 times, respectively). But the values of these ratios were similar in glycolipids and PG of *A. tobuchiensis*. The content of both major PUFAs were especially high in MGDG, where it reached equal levels-36.8%. This value was the highest among polar lipids not only of *A. tobuchiensis,* but also of other studied species of marine macrophytes (Tables 1–5). Thus, MGDG was the most unsaturated glycolipid of *A. tobuchiensis* in contrast to SQDG. Accordingly with UI and the ratio of saturated/unsaturated FAs, SQDG and PG were close by the level of saturation. As a whole, PG is closer to DGDG and SQDG by fatty acid composition. It is necessary to point out their common ability to form lamellar superstructure in contrast with non-lamellar MGDG (Garab et al., 2000).

Glycolipids of *L. japonica* are characterized by the same major fatty acids as phospholipids (Table 2). Thus, percentage of 16:0, 18:1n-9 and 18:2n-6 was substantial in all polar lipids of *L. japonica*. At that, the content of 16:0 strongly varied from 5.5 to 45.2% in glycolipids. The essential amount (3.6–9.0%) of other saturated FA, 14:0, also was observed in all glycolipids similar to phospholipids except for PG. On the contrary, the 18:0 was preferably accumulated in PG, where its content reached 23.0% against of 0.5–3.8% in other polar lipids. In turn, the largest amount of monoene acid 16:1 was occurred in DGDG (20.5%), while, in other glycolipids, its content was similar to percentage of PC and PE. Uncommon γ-linolenic acid 18:3n-6 and stearidonic acid 18:4n-3, both of which are commercially important (Callaway et al., 1996; Hong et al., 2002), were concentrated in MGDG. MGDG was also distinguished from other polar lipids of *L. japonica* by the highest content of 20:5n-3 (15.9%), which prevailed over the content of 20:4n-6 by 6%. Other glycolipids comprised only small amounts of both FAs. Probably, both PUFAs, especially 20:5n-3, play the particular roles as major constituents of algal MGDG. All photosynthetic polar lipids of *L. japonica* differed from non-photosynthetic ones by the contrary ratio of C_{18}/C_{20} PUFAs and by much less ratio of n-6/n-3 PUFAs. Thus, C_{18} PUFAs clearly dominated over C_{20} in glycolipids and PG in contrast to PC and PE of brown alga. In turn, the ratio of n-6/n-3 PUFAs was much higher in PC and PE in comparison with PG and glycolipids, especially with MGDG (4.6 and 5.6 against 0.6–2.2, respectively). The similar situation was observed in *A. tobuchiensis*. As a whole, MGDG was the most unsatd polar lipids in contrast to the most satd SQDG of *L. japonica*.

Other brown alga, *S. pallidum* belongs to the Fucales in contrast to *L. japonica,* representing the Laminariales. Taxonomic differences were reflected in fatty acid composition of their glycolipids (Table 4). So, *S. pallidum* was characterized by more diverse fatty acid composition of glycolipids in comparison with *L. japonica,* therefore percentages of FAs were more moderate in comparison both with *L. japonica* and *A. tobuchiensis*. Two additional acids, 17:0 and 16:4n-3, were appeared among major FAs, whereas 18:3n-6 should be excepted from this list due with its low content in glycolipids of *S. pallidum*. It was notable that 14:0, 16:0 and 16:1 are ca. equally distributed between glycolipids of *S. pallidum* in contrast to *L. japonica,* while the level of palmitic acid was ca. 4 times higher than 14:0 and 16:1. Other FAs essentially varied. It is necessary to point out the highest percentage of 17:0, 16:4n-6 and 18:0 (13.4, 8.8 and 5.4%, respectively) in SQDG and, on the other hand, the domination of linoleic acid (16.3%) in MGDG. In contrast to *L. japonica*, C_{18} PUFAs, 18:3n-3 and 18:4n-3, were preferably accumulated in DGDG, where they amounted 11.2 and 14.6%, respectively. Equal amounts of C_{20} PUFAs, 20:4n-6 and 20:5n-3, were concentrated in MGDG and DGDG (10–15%). The ratio of n-6/n-3 PUFAs did not exceed 1.6 similar to glycolipids of *L. japonica,* whereas C_{20} and C_{18} PUFAs were oppositely distributed in glycolipids of brown algae studied. The unsaturation level of glycolipids decreased in the common trend: MGDG→DGDG→SQDG.

Six major fatty acids were detected in glycolipids from green alga *U. fenestrata*. Among them, characteristic FA of green algae, 16:4n-3 was preferably accumulated in MGDG, where it reached of 43.7%. The equally high level of 18:4n-3 was revealed in MGDG and DGDG (*ca* 24%). The percentage of 18:3n-3 was significant in all glycolipids, while it was the highest in MGDG (24.1%). The largest amount of 18:2n-6 was found in DGDG (14.2% in contrast to 2.1% in other glycolipids). 16:0 was the sole major saturated FA of polar lipids of *U. fenestrata* where it was substantially concentrated in SQDG (58.7%) as well as in DGDG (ca. 25% in contrast to only 1% in MGDG). Also, SQDG was the most abundant in *cis*-vaccenic acid, which amounted more than 15% in contrast to 1.8 and 5% in MGDG and DGDG. 18:1n-7 is a characteristic FA of total lipids of green algae. Despite of different content of 18:1n-7, prevalence of this FA over 18:1n-9 was maintained in all glycolipids of *U. fenestrata*. PUFAs 20:4n-6 and 20:5n-3 were not detected in glycolipids, whereas ca. 2% of both C_{20} PUFAs contained in total lipids of *U. fenestrata* (Khotimchenko, 1993a).

Fatty acid composition of glycolipids from seagrass *Z. marina* was the simplest in comparison with macroalgae studied and was characteristic of higher terrestrial plants. Macrophytic algae differed from seagrasses, containing higher proportions of linoleic and α-linolenic acids in their total lipids (Dembitsky et al., 1991; Khotimchenko, 1993). As shown in Table 5, these prevailing fatty acids were differently distributed in phospholipids and glycolipids of *Z. marina*. Thus, in glycolipids, levels of 18:2n-6 and other major fatty acid, 16:0, were ca. 2–5 times lower, and, on the contrary, the percentage of 18:3n-3 was ca. 2 times higher in comparison with phospholipids. Phospholipids comprised equal contents of both C_{18} PUFAs, whereas 18:3n-3 significantly prevailed 18:2n-6 in both glycolipids (58.7% and 70% against ca. 10%, respectively). Especially homogeneous fatty acid composition of DGDG, dominating among glycolipids of *Z. marina* (Dembitsky et al., 1991), was resulted in cooperative thermal transition as we have shown earlier (Goncharova et al., 2002). It was the most interesting that C_{16} PUFA 16:3n-6, high level of which is characteristic of so-called C_{16} plants and of all representatives of Zosteraceae (Khotimchenko, 1993b), was completely accumulated in MGDG. In agreement with data of Khotimchenko (1993b), there were detected low amounts of C_{20} PUFA 20:5n-3 (1.2–1.9%) in all polar lipids except for PE of *Z. marina*, whereas traces of 20:4n-6 was revealed in phospholipids only.

2.3. Betaine lipid

Betaine lipid DGTS is important in green algae where it has been suggested to act as a substitute for PC in extra-chloroplast membranes (Harwood, 1998). Betaine lipids, DGTS and diacylglyceryltrimethyl-D-alanine (DGTA), were also found in thylakoid membranes of eukaryotic algae (Murata and Siegenthaler, 1998). Their role is unclear in algae cells. Previously, it has been assumed that the occurrence of this lipid is restricted to algae and lower plants (Sato, 1992). However, it is certainly now that during the course of evolution the ability to synthesize betaine lipids was not a newly acquired trait by eukaryotic algae, but had already evolved in photosynthetic bacteria (Benning, 1998). The non-phosphorus lipid DGTS was found to increase in *Rhodobacter sphaeroides* during growth under phosphate limitation. Therefore, DGTS was suggested to replace phospholipids under these conditions (Benning et al., 1995). The same relation was revealed in green macroalgae. The freshwater species usually have mainly PC and little DGTS while marine species have much more DGTS (Dembitsky, 1996). This may reflect the low availability of phosphate in the marine environment (Harwood, 1998).

Percentage of DGTS reaches 12% of total lipids from *U. fenestrate* (Table 6). It includes the same acyl residues as glycolipids or total lipids of this green alga (Khotimchenko, 1993a). Similar to DGDG and SQDG, DGTS contains the high amount of 16:0 prevailing 2 and more times other FAs. The content of C_{18} unsatd FAs (18:1n-7, 18:2n-6, 18:3n-3 and 18:4n-3) were also

significant in DGTS similar to glycolipids. However, there was also the essential level of 18:3n-6, 20:4n-6 and 20:5n-3 in DGTS (5.5, 4.9 and 3.5%, respectively) in contrast to glycolipids virtually not comprising them. In green algae, these three PUFAs are the typical components of total lipids, which comprises only low amounts of them as usual (1–2% in *U. fenestrata*) (Khotimchenko, 1993a). DGTS also differed from total lipids by the low content (less than 1%) of C_{16} PUFA 16:4n-3. In fact, high amount of 16:4n-3 was shown to be the distinguished trait of MGDG from *U. fenestrata* only.

3. Conclusions

The present study of fatty acid composition of polar lipid classes from marine macrophytes have indicated the important chemotaxonomic trait found out in the total lipids of these plants (Khotimchenko, 1999). Namely, the distribution of C_{18} and C_{20} PUFAs in major polar lipids of marine macrophytes depends from their taxonomic position. Thus, PUFAs of phospho- and glycolipids of red alga *A. tobuchiensis* mostly belonged to C_{20} series. In the contrary, the same lipids of seagrass *Z. marina* comprised predominantly C_{18} PUFAs, and polar lipids of brown alga *L. japonica* content high levels of both PUFA series. In spite of significant chemotaxonomic differences, polar lipids of all these species possess common traits of their FA composition, which can play the important adaptive role. So, the ratio between n-6 and n-3 PUFAs was far higher in phospholipids of non-photosynthetic membranes, PC and PE, compared with glycolipids and PG. n-6 PUFAs are appeared to be more functionally important in the content of the major lipids of plasma membranes. In algae, it was mainly occurred due with the most prevalence of 20:4n-6 over 20:5n-3 in non-photosynthetic lipids. As shown earlier, partial substitution of 20:5n-3 by 20:4n-6 in PC and PE of other marine poikiothermic organisms, invertebrates, related with thermoadaptive importance due with a different potent of n-6 and n-3 eicosanoids. Then, it should be not excluded that the same role is performed by these abundant PUFAs in marine macrophytes. The substitution of n-6 by n-3 PUFAs was accompanied by the partial substitution of C_{20} by C_{18} PUFAs in glycolipids and PG in contrast to PC and PE from *L. japonica*, where both series of PUFAs are presented.

The revealed similarity of FA composition of PG and glycolipids, especially, of SQDG, is agreed with the existence of common biosynthetic pathways of major polar lipids of thylakoid membranes. The saturation increasing in the lines of MGDG→DGDG→SQDG and PC→PE→PG. Many of characteristic PUFAs (16:4n-3, 16:3n-3, 18:3n-6 and 18:4n-3, 20:3n-6) appeared to be accumulated in MGDG of *U. fenestrata, Z. marina, L. japonica* and *S. pallidum,* respectively. Unusual 18:1n-7 was especially concentrated in PC of *A. tobuchiensis* and SQDG of *U. fenestrata*. DGTS of *U. fenestrata* differed by significant content of 18:3n-6, 20:4n-6 and 20:5n-3 in contrast to respective galactolipids. Percentage of 16:0 and 18:2n-6 was 2 and more times higher, whereas of 18:3n-3, on the contrary, was lower in phospholipids compared with glycolipids of seagrass. The set of major FAs of polar lipid classes can essentially differ as from each other and from total lipids of marine macrophytes.

4. Experimental

4.1. Algal material

5 species of marine macrophytes: *Anfeltia tobuchiensis* (Rodophyta), *Laminaria japonica* and *Sargassum pallidum* (Phaeophyta), *Ulva fenestrata (*Chlorophyta), *Zostera marina* (Embriophyta) were harvested in the Sea of Japan in summer at seawater temperature of 20–23 °C. Freshly collected algae or seagrass were thoroughly cleaned to remove epiphytes, small invertebrates and sand particles and then heated for 2 min in boiling H_2O to inactivate enzymes.

4.2. Lipid extraction and isolation

Total lipid extracts from ca. 10 kg of algae or seagrasses were obtained according to the method of Folch et al. (1957). Crude glyco- and phospholipids were isolated from total lipid extract by column chromatography on silica gel by elution with Me_2CO, Me_2CO/benzene/EtCOOH/H_2O (200:30:3:10, by vol.) and a gradient of $CHCl_3$ and MeOH. Then, lipids were purified by preparative silica TLC using Me_2CO/benzene/EtCOOH/H_2O (200:30:3:10, by vol.) and $CHCl_3$/MeOH/H_2O (65:25:4, by vol.). Purity of lipids was checked by two-dimensional silica TLC (Vaskovsky and Terekhova, 1979; Vaskovsky and Khotimchenko, 1982). Silica gel for TLC was prepared according to the method of Svetashev and Vaskovsky (1972).

4.3. GC analysis of fatty acid composition

Esterification of lipids was accomplished by the addition of freshly prepared acetylchloride–methanol (1:20) and leaving the reaction to take place at 95 °C for 1 h in the heating module Block-Therm (MTA KUTESZ, Hungary). Fatty acid Me esters were extracted with hexane and purified by TLC. Analysis of fatty acid Me esters was carried out using a gas–liquid chromatograph Shimadzu GC-9A, equipped with a flame-ionization detector, a silica capillary column (25 m×0.25 mm) with Carbowax 20M. The carrier gas was He. Individual

peaks of fatty acid Me esters were identified by comparison of GC R_tS with those of authentic standards of fatty acid Me esters and by ECL (equivalent chain length) (Kramer et al., 1985; Christie, 1988).

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References

Arao, T., Yamada, M., 1989. Positional distribution of fatty acids in galactolipids of algae. Phytochemistry 28, 805–810.

Benning, C., 1998. Membrane lipids in anoxygenic photosynthetic bacteria. In: Siegenthaler, P.-A., Murata, N. (Eds.), Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer Academic Publishers, Dordrecht, pp. 83–101.

Benning, C., Huang, Z.H., Gage, D.A., 1995. Accumulation of a novel glycolipid and betaine lipid in cells of *Rhodobacter sphaeroides* grown under phophate limitation. Arch. Biochem. Biophys. 317, 103–111.

Callaway, J.C., Tennilä, T., Pate, D.W., 1996. Occurrence of *"omega*-3" stearidonic acid (*cis*-6,9,12,15-octadecatetraenoic acid) in hemp (*Cannabis sativa* L.) seed. Journal of the International Hemp Association 3, 61–63.

Christie, W.W., 1988. Equivalent chain length of methyl ester derivatives of fatty acids on gas chromatography. J. Chromatogr. 447, 305–314.

Dembitsky, V.M., Pechenkina-Shubina, E.E., Rozentsvet, O.A., 1991. Glycolipids and fatty acids of some seaweeds and marine grasses from black sea. Phytochemistry 30, 2279–2283.

Eichenberger, W., Araki, S., 1993. Betaine lipids and phospholipids in brown algae. Phytochemistry 34, 1323–1333.

Goncharova, S.N., Sanina, N.M., Kostetsky, E.Y., 2000. Role of lipids in molecular thermoadaptation mechanisms of seagrass *Zostera marina*. Biochem. Soc. Trans. 28, 887–890.

Goncharova, S.N., 2003. Thermotropic behavior of major membrane lipids from marine macrophytes. PhD Thesis, Vladivostok [in Russian].

Gustafson, K.R., Cardellina, J.H., Fuller, R.W., Weislow, O.S., Kiser, R.F., Shader, K.M., Patterson, G.M., Boyd, M.R., 1998. AIDS-antiviral sulfolipids from cyanobacteria (blue-green alga). J. Natl. Cancer Institute 81, 1254–1258.

Harwood, J.L., 1998. Membrane Lipids in Algae. In: Siegenthaler, P.-A., Murata, N. (Eds.), Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer Academic Publishers, Dordrecht, pp. 53–64.

Hong, H., Datla, N., Reed, D.W., Covello, P.S., MacKenzie, S.L., Qiu, X., 2002. High-level production of γ-linolenic acid in *Brassica juncea* using a Δ6-desaturase from *Pythium irregulare*. Plant Physiology 129, 354–362.

Jones, A.L., Harwood, J.L., 1993. Lipid metabolism in the brown marine algae *Fucus vesiculosus* and *Ascophyllum nodosum*. J. Exp. Bot. 44, 1203–1210.

Joyard, J., Maréchal, E., Miège, C., Block, M.A., Dorne, A.-J., Douce, R., 1998. Structure, distribution and biosynthesis of glycoglycerolipids from higher plant chloroplast. In: Siegenthaler, P.-A., Murata, N. (Eds.), Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer Academic Publishers, Dordrecht, pp. 21–52.

Kayama, M., Araki, S., Sato, S., 1989. Lipids of marine plants. In: Ackman, R.G. (Ed.), Marine Biogenic Lipids, Fats and Oil. CRC Press Inc, Boca Raton, FL, pp. 3–48.

Kim, M.-K., Dubacq, J.-P., Thomas, J.-C., Giraud, G., 1996. Seasonal variation of triacylglycerols and fatty acids in *Fucus serratus*. Phytochemistry 43, 49–55.

Khotimchenko, S.V., 1993a. Fatty acids of green macrophytic algae from the Sea of Japan. Phytochemistry 32, 1203–1207.

Khotimchenko, S.V., 1993b. Fatty acids and polar lipids of seagrasses from the Sea of Japan. Phytochemistry 33, 369–372.

Khotimchenko, S.V., 1998. Fatty acids of brown algae from the Russian Far East. Phytochemistry 49, 2363–2369.

Khotimchenko, S.V., 1999. Distribution of polar lipids and fatty acids in marine macrophytes. PhD Thesis, Vladivostok [in Russian].

Khotimchenko, S.V., Vaskovsky, V.E., 1990. Distribution of C_{20} polyenoic fatty acids in red macrophytic algae. Botanica Marina 33, 525–528.

Kramer, J.K.G., Fouchard, R.C., Jenkins, K.J., 1985. Differences in chromatographic properties of fused silica capillary columns, coated, crosslinked, bonded, or crosslinked and bounded with polyethylene glycols (Carbowax 20M) using complex fatty acid methyl ester mixtures. J. Chromatogr. Sci. 23, 54–56.

Loya, S., Reshef, V., Mizrachi, E., Silberstein, C., Rachamim, Y.C.S., Hizi, A., 1998. The inhibition of the reverse transcriptase of HIV-1 by the natural sulfoglycolipids from cyanobacteria: contribution of different moieties to their high potency. J. Nat. Prod. 61, 891–895.

Marks, F., 1999. Arachidonic acid and companions: an abundant source of biological signals. In: Marks, F., Fürstenberger, G. (Eds.), Prostaglandins, Leukotrienes and Other Eicosanoids. From Biogenesis to Clinical Application. Wiley-VCH, Weinheim, pp. 1–46.

Melo, N., Tavares, R.M., Morais, F., Barroso, J.G., Pais, M.S.S., 1995. Lipid composition of thylakoid membranes from leaves and regreened spathes of *Zantedeschia aethiopica*. Phytochemistry 40, 1367–1371.

Morimoto, T., Nagatsu, A., Murakami, N., Sakakibara, J., Tokuda, H., Nishino, H., Iwashima, A., 1995. Anti-tumour-promoting glyceroglycolipids from the green alga, *Chlorella vulgaris*. Phytochemistry 40, 1433–1437.

Murata, N., Siegenthaler, P.-A., 1998. Lipids in photosynthesis: an overview. In: Siegenthaler, P.-A., Murata, N. (Eds.), Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer Academic Publishers, Dordrecht, pp. 1–20.

Ohta, K., Mizushina, Y., Hirata, N., Takemura, M., Sugawara, F., Matsukage, A., Yoshida, S., Sakaguchi, K., 1998. Sulfoquinovosyldiacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartinatenella*. Chem. Pharm. Bull. (Tokio) 46, 684–686.

Sanina, N.M., Goncharova, S.N., Kostetsky, E.Y., 2003. Seasonal changes in thermotropic behavior of phospho- and glycolipids from *Laminaria japonica*. In: Murata, N., Yamada, M., Nishida, I., Okuyama, H., Sekiya, J., Wada, H. (Eds.), Advanced Researches of Plant Lipids. Kluwer Academic Publishers, Dordrecht, pp. 385–388.

Sanina, N.M., Kostetsky, E.Y., 2002. Thermotropic behavior of major phospholipids from marine invertebrates: changes with warm-acclimation and seasonal acclimatization. Comp. Biochem. Physiol. 133B, 143–153.

Sanina, N.M., Kostetsky, E.Y., Goncharova, N.V., 2000. Thermotropic behaviour of membrane lipids from brown marine alga *Laminaria japonica*. Biochem. Soc. Trans. 28, 894–897.

Sanina, N.M., Kostetskii, E.Y., Shnyrov, V.L., 1987. Calorimetric investigation of phosphatidylcholine in membranes of marine invertebrates. Zh. Evol. Biochem. Physiol. 23, 451–460. [in Russian].

Sato, N., 1992. Betaine lipids. Bot. Mag. Tokyo 105, 185–197.

Sewón, P., Mikola, H., Lehtinen, T., Kallio, P., 1997. Polar lipids and net photosynthesis potential of subarctic *Diapensia lapponica*. Phytochemistry 46, 1339–1347.

Smith, K.L., Harwood, J.L., 1984. Lipids and lipid metabolism in the brown alga, *Fucus serratus*. Phytochemistry 23, 2469–2473.

South, G.R., Whittick, A., 1987. Introduction to Phycology. Blackwell Scientific Publications, Oxford.

Svetashev, V., Vaskovsky, V.E., 1972. A simplified technique for thin-layer microchromatography of lipids. J. Chromatogr. 67, 376–378.

Tarchevsky, I.A., 2002. Plant Cell Signaling Systems. Nauka, Moscow [in Russian].

Thompson, G.A., 1996. Lipids and membrane function in green algae. Biochim. Biophys. Acta 1302, 17–45.

Vaskovsky, E.V., Khotimchenko, S.V., 1982. HPTLC of polar lipids of algae and other plants. J. Chromatogr. 5, 635–636.

Vaskovsky, E.V., Khotimchenko, S.V., Xia, B., Heffang, L., 1996. Polar lipids and fatty acids of some marine macrophytes from the Yellow Sea. Phytochemistry 42, 1347–1356.

Vaskovsky, E.V., Terekhova, T.A., 1979. HPTLC of phospholipids mixtures containing phosphatidylglycerol. J. High Resol. Chromatogr. 2, 671.

Williams, J.P., Khan, M.U., Wong, D., 1996. Fatty acid desaturation in monogalactosyldiacylglycerol of *Brassica napus* leaves during temperature acclimation. Physiologia Plantarum 96, 258–262.