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Response of prostaglandin content in the red alga *Gracilaria verrucosa* to season and solar irradiance

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Abstract

The influence of solar irradiance and seasons on prostaglandin (PG) and arachidonic acid (AA) content in the marine red alga *Gracilaria verrucosa* (Huds.) Papenf. (unattached form) was investigated. PGA_2, PGE_2, PGF_2, and 15-keto-PGE_2 were isolated from the alga, quantitatively analyzed as 4-methyl-7-methoxycoumarin esters by high-performance liquid chromatography, and their chemical structures were confirmed by ^1H NMR. In June–September, the PG content in the alga was relatively stable (420 μg/g of dry wt. of $PGE_2 + PGF_2$; 40 μg/g of PGA_2) and it increased 1.5 times in October. The highest level of PGs was detected in November (2500 μg/g of $PGE_2 + PGF_2$; 74 μg/g of PGA_2) when water temperature was fairly low (5–10 °C). Algae grown for five months at 50% of incident photosynthetic active radiation (PAR_0) contained two times less PGE_2 and PGF_2 than algae grown under natural conditions, but the amount of these PG in algae grown at 5% of PAR_0 was close to the normal level. On the contrary, when algae were grown at 5% of PAR_0 the content of PGA_2 increased up to 4 times compared to algae cultivated at 100% PAR_0. In June–November, the amount of AA in total algal lipids slightly varied from 48.9 to 56.7% and did not virtually depend on the light intensity. The probable reasons of the PG content variation in response to environmental factors are discussed.

Keywords: *Gracilaria verrucosa*; Solar irradiance; Environmental conditions; High-performance liquid chromatography; Gas chromatography; NMR; Prostaglandins; Arachidonic acid; 4-Bromomethyl-7-methoxycoumarin

1. Introduction

Red algae are rich in polyunsaturated fatty acids (PUFA) with 20 carbon atoms, mainly arachidonic (AA) and eicosapentaenoic acids, which are precursors of prostaglandins (PG). It has been demonstrated that marine plants are capable of metabolizing C_{20} PUFA to eicosanoids via various oxidative pathways. Oxidized fatty acid derivatives were isolated from fairly many species of Rhodophyta (Gerwick and Bernart, 1993). Algae of the genus *Gracilaria*, as well as *Gracilariopsis*, are of particular interest because they contain eicosanoids characteristic of the higher plants and human. Acyclic eicosanoids such as 12-HETE and 12-HEPE were found in *Gracilariopsis lemeneiformis* (Jiang and Gerwick, 1991). Two isomers of LTB_4 (6-*trans*-LTB_4 and 6-*trans*-12-*epi*-LTB_4,) as well as 8-HETE were detected in *Gracilaria asiatica* (Sajiki and Kakimi, 1998; Sajiki, 1999). However, members of the genus *Gracilaria* are distinguished from other red algae by that they contain PG. Thus, PGE_2 and PGF_2 were isolated from *Gracilaria lichenoids* (Gregson et al., 1979); PGE_2 and 15-keto-PGE_2 were found in *G. asiatica* (Sajiki, 1999). *Gracilaria verrucosa* was found to contain PGA_2 in addition to PGE_2; however, its presence in the alga may be a consequence of partial non-enzymatic dehydration of PGE_2 (Fusetani and Hashimoto, 1984). These PG from Japanese *G. verrucosa* appear to be responsible for a gastro-intestinal disorder, known as ''ogonori'' poisoning, which is associated with ingestion of the popular edible seaweeds in Japan (Fusetani and Hashimoto, 1984).

The most widespread *G. verrucosa* in the Sea of Japan is the attached form of alga growing on a rocky substrate. However, there is an unattached form of the same species found in lagoons, which differs from the

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attached form by that it reproduces asexually, by fragmentation (Tityanova et al., 1990). The unattached form contains much more agar and twice as much lipids as the attached one (Lapshina et al., 1993; Khotimchenko and Levchenko, 1997).

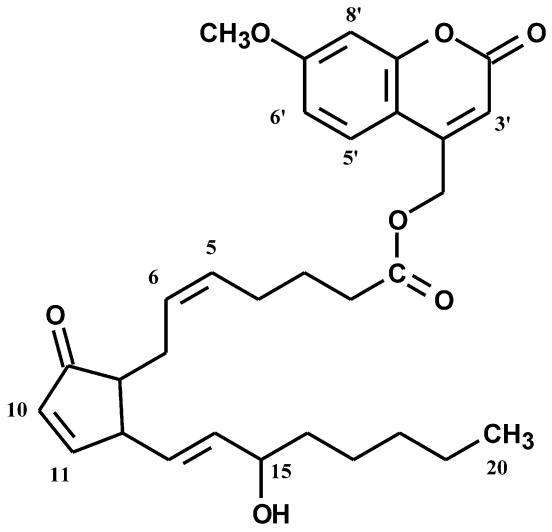
The aim of the present study was to determine the PG composition and the influence of environmental factors such as season and solar irradiance on the PG content in the unattached form of *G. verrucosa*. In addition, we have investigated the relation between contents of AA and PG in this alga.

2. Results and discussion

2.1. Identification of PG in the alga and optimization of HPLC analytical method

We identified prostaglandins PGA_2, PGE_2, PGF_2, and 15-keto-PGE_2 as major eicosanoids in the unattached form of red alga *G. verrucosa*. The presence of these compounds in the form of methyl esters was confirmed by co-chromatography with the authentic PG standards using TLC and HPLC. It was shown that at least 25 mg PGE_2 methyl ester, 8 mg 15-keto-PGE_2 methyl ester, and 20 mg PGF_2 methyl ester can be isolated from 1 kg of fresh *G. verrucosa* using a combination of chromatographic methods (Nevshupova and Imbs, 1999). The structure of PGE_2, PGF_2, and 15-keto-PGE_2 was unequivocally proved by analysis of their ^{1}H NMR spectra which were identical to those of authentic PG methyl esters and the spectra reported previously (Schneider et al., 1977; Fusetani and Hashimoto, 1984). Unfortunately, the quantity of obtained PGA_2 methyl ester was not sufficient for correct NMR analysis. The presence of PGF_2 in the attached form of *G. verrucosa* has not been reported in recent works (Sajiki and Kakimi, 1998; Sajiki, 1999).

To determine the quantity of PG in *G. verrucosa* samples, we applied the HPLC analysis of their 4-bromomethyl-7-methoxycoumarin (BrMMC) derivatives. Firstly, a semi-preparative amount of BrMMC derivatives of individual PGB_2, PGD_2, PGA_2, PGE_2, and $PGF_{2\alpha}$ was synthesized with a yield more than 80%. We applied the reaction between organic acid salt and BrMMC in the presence of a phase-transfer catalyst in non-aquiver solvent as described earlier (Tsuchiya et al., 1982; Berger and Petz, 1991) with some variances. Acetonitrile usually used as the solvent was partly replaced with chloroform to increase the solubility of BrMMC and the lipid extract of *G. verrucosa*. The optimum temperature was 50–55 °C and time 50–60 min. The structure of the synthesized compounds was confirmed by the ^{1}H NMR analysis. As an example, the structure of (4′-methylene-7′-methoxycoumarin)-PGA_2 ester (**1**) and selected ^{1}H NMR signal assignments (Table 1) are presented. The spectrum showed a terminal methyl signal at δ 0.89 (*t*), one carbinol methine at δ 4.09 (*m*), three sets of double bound at δ 5.40, 5.43, 5.61, 5.70, and a methylene adjacent to a carboxyl group at δ 2.32. The values of 5, 6, and 16 Hz coupling constants are typical of the $\Delta5(Z)$ and $\Delta13(E)$ olefin functionalities. A set of double bound at δ 6.18, 7.50 suggested the presence of α,β-unsaturated cyclopentenone. These features were quite similar to those of PGA_2 ester. The spectrum also showed signals of four coumarin protons at δ 6.33, 6.84, 6.99, 7.42 and a methoxy group at δ 3.66 (*s*) (Table 1). The ^{1}H NMR spectra of other synthesized compounds contained the described signals of the methylmethoxycoumarin group and signals similar to ^{1}H NMR shifts of PG esters published elsewhere (Schneider et al., 1977; Chen and Janda, 1997, 1998; Mikolajczyk et al., 2000).



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An isocratic reversed-phase HPLC separation of the BrMMC derivatives of the five synthesized PG was achieved within 30 min in a single run. The peaks of esters were completely resolved when a 25-cm column was used. With a 15-cm column, the resolution of PGE_2 and PGF_2 was poorer, but the analysis time was reduced to 16 min. As a rule, we determined the sum of PGE_2 and PGF_2 because many tests on a 25-cm column showed that the amount of PGE_2 related to that of PGF_2 as 25:10 and this value was quite stable in all experiments.

Fusetani and Hashimoto (1984) successfully used hot methanol to extract PG from *G. verrucosa*. Our experiments showed that the heating time strongly influences the content of PG in the prepared extract. The maximum content of PG was isolated from alga after 5 min heating, but the PG level fell drastically after 1 h heating (Table 2). A temporary increase in the amount of PGA_2 in 30 min was most likely caused by non-enzymatic

Table 1
Selected ^{1}H NMR spectra assignments for (4′-methylene-7′-methoxycoumarin)PGA_2 ester (**1**) (250 MHz, $CDCl_3$)

H	δ (ppm/TMS)	J (Hz)
3	6.33 *t*	1.5
5	7.42 *d*	9.5
6	6.99 *d*	2.5
8	6.84 *s*	
2	2.32 *t*	7
3	1.87 *m*	
4	2.09 *m*	
5	5.40 *t*	5
6	5.43 *t*	5
10	7.50 *dd*	6, 2
11	6.18 *dd*	6, 2
13	5.61 *dd*	16, 6
14	5.70 *dd*	16, 6
15	4.09 *m*	
OMe	3.66 *s*	
17,18,19	1.20–1.35 *m*	
20	0.89 *t*	6

Table 2
Changes in the prostaglandins content in methanol extract of *G. verrucosa* (unattached form) at heating (55 °C)

Heating time (min)	Content of prostaglandins in algal extract (μg/g dry wt.)		
	15-keto-PGE_2	$PGE_2 + PGF_2$	PGA_2
5	90	398	40
30	47	307	78
60	14	115	22

dehydratation of PGE_2. A long-period soaking of *G. verrucosa* (up to 48 h) in methanol at room temperature recovered PG with a very low yield. The classic method of total lipids extraction with chloroform–methanol was not suitable because of chromatographic difficulties in detecting extremely low levels of PG in algal total lipid extract.

It should be noted that our attempts at the direct HPLC analysis of crude reaction mixture after derivatization with BrMMC were unsuccessful. To remove an excess of BrMMC and non-polar substances, we purified BrMMC derivatives on a solid-phase extraction tube with silica gel. All PG esters were quantitatively recovered from the tube without appreciable losses.

Thus, high-performance liquid chromatography of the BrMMC derivatives of PG has proven to be a powerful method for the analysis of eicosanoids in marine algae. Using this method, we obtained new data on the variation of *G. verrucosa* PG content in relation to season and light intensity.

2.2. Changes of PG and AA content related with seasons and solar irradiance

In the lagoons of southern Primorye, the unattached form of *G. verrucosa* begins to grow in May. Growth is highest in July–August; in September–October it is markedly retarded, and fragmentation of the thalli commences. In December–March, alive alga winters frozen into the ice (Tityanova et al., 1990).

In June–September 1999, the unattached form of *G. verrucosa* did not show significant differences in the levels of 15-keto-PGE_2, PGE_2, and PGF_2 but there were higher levels of the PG in algae collected in October (Table 3). The highest level of the above PG was detected in November when water temperature is relatively low (5–10 °C). In contrast, no significant difference ($P > 0.05$) in the PGA_2 content of *G. verrucosa* was found in June-November (Table 3). Measurements of the levels of PG in *G. verrucosa* were repeated at the next year (2000) and the results did not differ significantly ($P < 0.05$, data not shown). Recently, an appreciable increase in the PGF_2 level in February compared to July was described for the attached form of *G. asiatica* growing in the Bay of Tokyo, Sea of Japan (Sajiki, 1997).

A variety of factors can be responsible for the accumulation of PG in the cold season: environmental conditions (solar irradiance, seawater temperature, contents of dissolved components, etc.), level of the PG biosynthesis substrate (arachidonic acid) in algal tissues, activity of enzymes of eicosanoid synthesis, stage of life cycle, and others.

We found that the level of PG in the unattached form of *G. verrucosa* depends on solar irradiation. The content of PG in algae cultivated during 5 months under

Table 3
Changes in the content of prostaglandins (μg/g dry wt.) in *G. verrucosa* (unattached form) during June–November

Prostaglandin	Collection time				
	June	July	September	October	November
15-keto-PGE_2	90 ± 6^a	86 ± 18^a	120 ± 42^a	$231 \pm ^b$	306 ± 18^c
$PGE_2 + PGF_2$	398 ± 21^a	413 ± 45^a	429 ± 64^a	640 ± 43^b	2468 ± 112^c
PGA_2	40 ± 4^a	47 ± 14^a	46 ± 12^a	67 ± 17^a	74 ± 4^a

Data are mean ± S.D. of triplicates. Different superscript letters in the same measurements are significantly different ($P < 0.01$) among the same prostaglandin.

Table 4
Changes in the content of prostaglandins (μg/g dry wt.) in *G. verrucosa* (unattached form) grown during June-October at various levels of photosynthetic active radiation (PAR_0)

Prostaglandin	PAR_0		
	95%	50%	5%
15-keto-PGE_2	231 ± 3^a	113 ± 12^b	213 ± 15^a
PGE_2+PGF_2	640 ± 43^a	332 ± 52^b	496 ± 1^c
PGA_2	67 ± 17^a	122 ± 20^b	226 ± 27^c

Data are mean ± S.D. of triplicates. Different superscript letters in the same measurements are significantly different ($P<0.05$) among the same prostaglandin.

different light intensity are shown in Table 4. The amount of 15-keto-PGE_2, PGE_2, and PGF_2 in algae exposed to 50% of incident photosynthetic active radiation (PAR_0) was about half that of the control sample ($P<0.01$), but the quantity of 15-keto-PGE_2 was relatively stable and the level of PGE_2 and PGF_2 were slightly lower ($P<0.05$) in algae grown under extremely low illumination conditions (5% PAR_0). On the contrary, the decreasing light intensity led to a consistent increase in PGA_2 in the algae ($P<0.05$). Hence, it is highly improbable that the reduced solar irradiance in October-November can cause the observed high level of main PG (except for PGA_2) in *G. verrucosa* in the cold period. Conceivably, the mechanism of formation of PGA_2 in *G. verrucosa* differs from that of the other PG, and part of PGA_2 originated from PGE_2 by a non-enzymatic process which intensified under adverse environmental conditions.

The changes in the fatty acid composition of a number of *Gracilaria* species cultivated under different environmental conditions were previously investigated, and it was shown that the arachidonic acid is the dominant fatty acid (Khotimchenko and Levchenko, 1997). Our data on *G. verrucosa* are indicative only of a slight increase in AA content (Table 5) in total algal lipids in the cold period, as well as under reduced solar irradiance (data not shown), and confirm the conclusion that the increase in AA content due to low temperature and low light intensity, which has been demonstrated in higher plants, is not consistent in *G. verrucosa* (Dawes et al., 1993).

Table 5
Content of arachidonic and eicosapentaenoic acids (% of total fatty acids) in *G. verrucosa* (unattached form) in June–November

Collection time	Content of fatty acids (%)		Seawater temperature (°C)
	Arachidonic	Eicosapentaenoic	
June	48.9	0.9	17
July	49.2	1.1	20
August	46.6	0.5	22
September	49.6	0.5	18
October	45.9	5.5	10
November	56.7	1.6	5

There was only a slight increase in AA content in November which did not correlate with a 6-fold rise in PG amount in the alga. On the other hand, the inhibition of PG synthesis in *G. verrucosa* by a high level of AA was postulated earlier (Sajiki, 1997). Thus, it is improbable that the seasonal changes in PG content in the unattached form of *G. verrucosa* were regulated by the AA level in algae lipids.

It is also unlikely that the total level of PG in *G. verrucosa* is dependent on the mode of reproduction because both the attached form, which reproduces sexually, and the unattached form, which reproduces vegetatively way, have markedly higher PG content in the summer than in the winter.

It should be noted that the PG content in the attached form of *G. verrucosa* collected at the same site was twice lower (207 ± 14 μg/g dry wt. of PGE_2+PGF_2, July sample) compared to the unattached one. This difference in PG content can be attributed to that the unattached form of the alga synthesized and accumulated twice as much lipids as the attached one, 5.6 and 2.8 mg/g, respectively (Khotimchenko and Levchenko, 1997).

The major lipid classes of *Gracilaria* that contain AA are monogalactosyldiacylglycerol (75% of AA), and phosphatidylcholine (73% of AA) (Araki et al., 1990). Since neither phospholipase A_1 nor A_2 activity was detected in raw alga (Sajiki, 1997), the splitting-off of arachidonic acid by acylhydrolases from monogalactosyldiacyl glycerol can be considered as a probable source of the substrate for PG synthesis in *G. verrucosa*. In this event, the PG level in *G. verrucosa* can be modulated through regulation of the activity of acylhydrolases or enzymes of the AA oxidation cascade.

It is most likely that the increased PG level in *G. verrucosa* is a response to decreased water temperature in the fall-winter period and it is not related to reduced solar irradiance. *G. verrucosa* is the rare algal species that contains PG and the level of PUFA in this alga is little dependent on water temperature, so we can postulate that PG may be involved in processes allow the algal tissue to survive in cold water and ice.

3. Experimental

3.1. Study site and sampling

Samples of *G. verrucosa* (unattached form) were collected during June–November 1999 in lagoons of southern Primorye (Peter the Great Bay, the Sea of Japan). Algae were grown in lagoons by the method of "rope culture" (Titlyanov et al., 1995) from June through October under natural light at 95% of incident

photosynthetic active radiation (PAR_0). Irradiance level was maintained using neutral density filters (50 and 5% PAR_0) and determined with an LI-190SA flat quantum sensor (LI-Cor, Lincoln, Nebraska). All samples were immediately frozen and stored at −20 °C not more than 1 week before analysis.

3.2. Isolation of PG methyl esters from G. verrucosa

Frozen alga (347 g) was extracted with $CHCl_3$–MeOH (1:2), the total lipid extract (730 mg) was redissolved in 10 ml of Et_2O, and 20 ml of saturated solution of diazamethane in Et_2O at 0 °C was added to obtain carboxylic acid methyl esters. After 1 h, the solution was evaporated and the residue was separated by low pressure silica gel column chromatography with gradient from $CHCl_3$ to MeOH. Fractions containing total PG methyl esters were additionally separated using a gradient of C_6H_6–EtOAc. Finally, pure PGE_2 methyl ester (9 mg), 15-keto-PGE_2 methyl ester (3 mg) and PGF_2 methyl ester (7 mg) were isolated by preparative HPLC with MeOH–H_2O (4.5 ml min^{-1}) on a Zorbax ODS column (25 cm×9.4 mm i.d.) with a refractive index detector. TLC of the obtained PG methyl esters was carried out on silica gel plates with C_6H_6–EtOAc (1:1); analytical HPLC was performed on a Zorbax ODS column (25 cm×4.6 mm i.d.) with UV detectors (200 nm) in MeOH–H_2O (65:35) at a flow rate of 1 ml min^{-1}. ^1H NMR spectra of PG esters were recorded at 250 MHz in $CDCl_3$.

3.3. Synthesis of 4′-methylen-7′-methoxycoumarin esters of PG

The work solutions of 21 mg dicyclohexyl-18-crown-6 (DCHC) in 0.2 ml CH_3CN and 15 mg 4-bromomethyl-7-methoxycumarin (BrMMC) in a mixture of 0.1 ml CH_3CN and 0.9 ml $CHCl_3$ were prepared. 1 mg PG was transferred with 0.1 ml $CHCl_3$ into a 1 ml reaction vial with a screw cap. After adding 0.05 ml of the DCHC solution, 0.2 ml of the BrMMC solution, and 0.1 g K_2CO_3, the PG reacted for 1 h at 55 °C. The reaction mixture was filtered, evaporated, and the PG ester was purified by TLC in $CHCl_3$–MeOH (9:1). BrMMC derivatives of PGB_2, PGD_2, PGA_2, PGE_2, and $PGF_{2\alpha}$ were synthesized. ^1H NMR spectra of PG derivatives were recorded at 250 MHz in $CDCl_3$.

3.4. Analysis of the PG content in G. verrucosa

About 120 g of freshly frozen alga were homogenized with 200 ml of MeOH, heated to 60C, and after 5 min filtered under vacuum. The solution was evaporated to a volume of 50 ml, acidified to pH 2, and extracted twice with 50 ml of EtOAc. Evaporation to dryness of combined extracts resulted in 97 mg of polar lipids. About 30 mg of the polar lipids were transferred with 0.2 ml $CHCl_3$ into a 1 ml reaction vial with a screw cap. After adding 0.1 ml CH_3CN, 0.05 ml of the DCHC solution, 0.2 ml of the BrMMC solution, and 0.2 g of K_2CO_3, the mixture was heated at 55 °C for 1 h. The reaction mixture was filtered, dried, dissolved in C_6H_6–EtOAc (85:15), and placed on a 3 ml solid-phase extraction tube with silica gel. The tube was washed with 2 ml of the same system, then the crude PG esters were eluted with 3 ml $CHCl_3$–MeOH (1:1). The last fraction was evaporated, solved in 0.2 ml MeOH and a 10 μl portion was analyzed by HPLC on a Zorbax ODS column (15 cm×4.6 mm i.d.) with a UV detector at 313 nm. An isocratic system of MeOH–H_2O (70:30) at a flow rate of 1.5 ml min^{-1} was used. The quantity of PG esters was calculated using calibration graphs of peak areas of PG esters standards synthesized previously.

3.5. Fatty acid (FA) analysis

Lipids were extracted according to Bligh and Dyer (1959). Fatty acid methyl esters (FAME) of total lipids were prepared according to Carreau and Dubacq (1979) and purified by TLC in C_6H_6. Analysis of FAME was carried out on a GC equipped with a flame-ionization detector, a fused quartz capillary column (30 m×0.25 mm i.d.) with Supelcowax 10M; split ratio 1:30, carrier gas He (1 ml min^{-1}). The column and detector temp was 210°, the injector temp was 240°. Identification of FAME was confirmed by chromatographic comparison with authentic standards and calculation of ECL (Christie, 1988).

3.6. Statistical analysis

Statistical analysis of the data was carried out as described elsewhere (Friedlander et al., 1993). Significant differences were located using Duncan's new multiple range test (one-way ANOVA's) and Student's *t*-test.

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References

Araki, S., Sakurai, T., Oohusa, T., Kayama, M., Nisizawa, K., 1990. Content of arachidonic and eicosapentaenoic acids in polar lipids from *Gracilaria* (Gracilariales, Rhodophyta). Hydrobiologia 204, 513–519.

Berger, K., Petz, M., 1991. Fluorescence HPLC determination of penicillins using 4-bromomethyl-7-methoxycoumarin as pre-column labelling agent. Deutsche Lebensmittel-Rundschau 87, 137–141.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Carreau, J.P., Dubacq, J.P., 1979. Adaptation of macro-scale method to the micro-scale for fatty acid methyl transesterification of biological lipid extracts. J. Chromatogr. 151, 384–390.

Chen, S.Q., Janda, K.D., 1997. Synthesis of prostaglandin E-2 methyl ester on a soluble- polymer support for the construction of prostanoid libraries. J. Am. Chem. Soc. 119, 8724–8725.

Chen, S.Q., Janda, K.D., 1998. Total synthesis of naturally occurring prostaglandin F-2 alpha on a non-cross-linked polystyrene support. Tetrahedron Lett. 39, 3943–3946.

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

Dawes, C.J., Kovach, C., Friedlander, M., 1993. Exposure of *Gracilaria* to various environmental conditions. II. The effect on fatty acid composition. Botanica Marina 36, 289–296.

Friedlander, M., Dawes, C., Levy, I., 1993. Exposure of *Gracilaria* to various environmental conditions. II. The effect of growth. Botanica Marina 36, 283–288.

Fusetani, N., Hashimoto, K., 1984. Prostaglandin E_2 — a candidate for causative agent of ogonori poisoning. Bull. Jpn. Soc. Sci. 50, 465–469.

Gerwick, W.H., Bernart, M.W., 1993. Eicosanoids and related compounds from marine algae. Marine Biotechnology 1, 102–152.

Gregson, R.P., Marwood, J.F., Quinn, R.J., 1979. The occurrence of prostaglandins PGE2 and PGF2-alfa in a plant—the red alga *Gracilaria lichenoides*. Tetrahedron Lett. 46, 4505–4506.

Jiang, Z.D., Gerwick, W.H., 1991. Eicosanoids and other hydroxylated fatty acids from the marine alga *Gracilariopsis lemaneiformis*. Phytochemistry 30, 1187–1190.

Khotimchenko, S.V., Levchenko, E.V., 1997. Lipids of the red alga *Gracilaria verrucosa* (Huds.) Papenf. Botanica Marina 40, 541–545.

Lapshina, A.A., Belokopytov, S.V., Ivanova, E.G., Tityanov, E.A., 1993. Agar concentration and properties in the unattached form of *Gracilaria verrucosa* depending on thalli growth intensity and habitat conditions. Russian J. Marine Biology 5-6, 107–117.

Mikolajczyk, M., Mikina, M., Jankowiak, A., 2000. A novel synthesis of racemic and enantiomeric forms of prostaglandin B-1 methyl ester. Journal of Organic Chemistry 65, 5127–5130.

Nevshupova, N.V., Imbs, A.B., 1999. Prostaglandin composition of red alga *Gracilaria verrucosa*. Russian J. Marine Biology 25, 142–143.

Sajiki, J., 1997. Effects of acetic acid treatment on the concentrations of arachidonic acid and prostaglandin E2 in the red algae, *Gracilaria asiatica* and *G. rhodocaudata*. Fisheries Sci. 63, 128–131.

Sajiki, J., Kakimi, H., 1998. Identification of eicosanoids in the red algae, *Gracilaria asiatica*, using high-performance liquid chromatography and electrospray ionization mass spectrometry. J. Chromatogr. 795, 227–237.

Sajiki, J., 1999. Acid treatment increased leukotriene B4 in the red alga, *Gracilaria asiatica* (= *verrucosa*). Fisheries Sci. 65, 914–918.

Schneider, W.P., Bundy, G.L., Lincoln, F.H., Daniels, E.G., Pike, J.E., 1977. Isolation and chemical convertions of prostaglandins from *Plexaura homomalla*: preparation of prostaglandin E_2, prostaglandin $F_2\alpha$, and their 5,6-*trans* isomers. J. Am. Chem. Soc. 99, 1222–1232.

Tityanova, T.V., Titlyanov, E.A., Kozmenko, V.B., 1990. Unattached form of *Gracilaria verrucosa* in lagoons of the southern Primorye. Russian J. Marine Biology 15, 45–50.

Titlyanov, E.A., Tityanova, T.V., Skriptsova, A.V., 1995. First experimental cultivation of unattached form of an agariferous alga *Gracilaria* in Russia. Russian J. Marine Biology 21, 146–156.

Tsuchiya, H., Hayashi, T., Naruse, H., Takagi, N., 1982. Sensitive high-performance liquid chromatographic method for prostaglandins using a fluorescence reagent, 4-bromomethyl-7- acetoxycoumarin. J. Chromatogr. 231, 247–254.