The major changes in lipid composition of *Sargassum horneri* during different growth phases

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distinctive and specialized group of marine animals and subtidal algae (Nanba 1995; Choi et al. 2003). The maturation of *S. horneri* occurs from winter to spring (Zeng and Lu 2000). It is believed that the seasonal shift in *S. horneri* maturation along the Chinese coast is caused by the temperature gradient of coastal water along the latitude. Egg release and germling development are very important for this species because algae are renewed annually through a new generation of germlings (Nanba 1995).

To date, many phycologists have examined the life cycle and reproduction period of *S. horneri* (Uchida 1993; Yoshida et al. 2001; Xie et al. 2014); however, the changes in lipid composition of *S. horneri* during different growth phases (from germlings to mature stages) remain largely unexplored. Eukaryotic membranes contain a diverse group of lipid molecular species, and the lipid composition alters in response to both internal and external cues. *Sargassum horneri* may possess different lipid compositions during various growth stages, since they grow during different seasons in the field. Therefore, we aimed to examine changes in membrane lipid composition of *S. horneri* during different growth phases, and to determine the physiological differences among these stages.

Materials and methods

Chemicals and reagents

Acetonitrile, isopropanol, formic acid, tetrahydrofuran, lithium acetate, and leucine-enkephalin were of liquid chromatography-mass spectrometry (LC-MS) grade from Sigma-Aldrich (USA). Pure water was purified using a Milli-Q system (Millipore, USA). Glycolipid standards, including MGDG, DGDG, and SQDG, were supplied by Lipid Products (UK). Diacylglyceryl-N,N,N-trimethylhomoserine (DGTS) was provided by Avanti Polar Lipids (USA).

Plant materials and cultivation treatments

The marine alga *S. horneri* was cultured from May to May of the coming year in a cement pool under constant aeration in Zhejiang Mariculture Research Institute (China). The seawater (salinity of 26–33 PSU) was prepared through sand filtration prior to the algae culture. The seawater depth in the cement pool was 1 m, and the seawater was refreshed every 2 days. The average temperature and photon flux density of every month are shown in Table S1. Algal specimens were collected on 28 September (young sporophyte), 30 November (sporophyte) in 2014, and 20 May (mature sporophyte) in 2015. Nutrients of N (about 10 mg L^{-1}) and P (about 1 mg L^{-1}) were supplemented every day for the growth of *S. horneri* (Fig. 1). All experiments were performed in five replicates and reported as mean ± standard deviation (SD). At the end of the experiment, the algae were frozen in liquid nitrogen and stored at −80 °C prior to further analysis.

Lipid analysis

Briefly, 50 mg of algae (freeze-dried) was extracted according to a previously established procedure by Bligh and Dyer (1959). The sample was dried under nitrogen gas and then dissolved in 0.5 mL methanol for ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-qTOF-MS) analysis.

Chromatographic separation was performed on an ACQUITY UPLC BEH C8 analytical column (100 × 2.1 mm, 1.7 μm, Waters, USA) using an ACQUITY UPLC system (Waters). Optimal separation was achieved with a gradient elution using (A) a mixture of acetonitrile/water (2:1, *v*/*v*/*v*, containing 0.1% formic acid and 0.01% lithium acetate, *v*/*v*) and (B) a mixture of acetonitrile/isopropanol/tetrahydrofuran (1:1:1, *v*/*v*/*v*, containing 0.1% formic acid and 0.01% lithium acetate, *v*/*v*) at a flow rate of 0.5 mL min^{-1}. The gradient elution program (time, min, %B) was set as follows: (0, 2); (3, 2); (5, 30); (12, 50); (22, 80); (25, 80); (27, 2). The temperature of sample chamber was maintained at 4 °C, the column temperature was set at 40 °C, and the injection volume was 3 μL for each analysis. Samples were filtered through a 0.22-μm ultra filtration membrane (Millipore, USA) prior to the injection.

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test). The lipid content in *S. horneri* was assessed by ANOVA. Results are presented as the mean ± SD. $P < 0.05$ was considered statistically significant. Analysis was undertaken using SPSS 13.0 for Windows. The lipids (MGDG, DGDG, and SQDG) were identified according to previously described protocols by Wang et al. (2014) and Li et al. (2014). Unfortunately, DGTAs are not commercially available at all, and no previous studies have characterized the location of fatty acyl in DGTA. Therefore, in this report, the distribution of fatty acyl chains in DGTA was characterized according to conclusions drawn by others (Roche and Leblond 2010). In addition, its semi-quantitative analysis was completed according to the ratio of the area of each DGTA molecule to that of its DGTS standard since both DGTA and DGTS are structural analogs.

Results

Profiling of lipid species in *S. horneri*

We characterized the lipid composition of *S. horneri* at different growth stages, mainly including photosynthetic glycerolipids (MGDG, DGDG, and SQDG) and the betaine lipid DGTA, using UPLC-qTOF-MS (Table 1). Eight MGDGs, 1 DGDG, 18 SQDGs, 1 lyso-SQDG, and 15 DGTAs were identified in *S. horneri* (Table 1). The proportion of each class in total lipids (nmol g^{-1} dry weight) is reported in

Table

Table 1 Content of lipid molecular species in *S. horneri*. Different letters indicate significant differences (Dunnett's test, $P < 0.05$) during different growth phases of *S. horneri*

Identification	Young sporophytes (nmol g^{-1})	Sporophytes (nmol g^{-1})	Mature male sporophytes (nmol g^{-1})	Mature female sporophytes (nmol g^{-1})
MGDG (18:4/18:4)	754.4 ± 1.9a	912.7 ± 14.1b	528.1 ± 12.5c	518.4 ± 54.3c
MGDG (20:5/18:4)	1356.6 ± 37.8a	1406.3 ± 61.3a	1192.7 ± 18.2b	1039.0 ± 53.7c
MGDG (20:5/18:3)	728.9 ± 35.0a	267.4 ± 8.2b	370.7 ± 4.8c	266.7 ± 18.4b
MGDG (14:0/18:2)	184.0 ± 8.3	177.7 ± 10.5	180.0 ± 12.0	163.4 ± 23.2
MGDG (16:0/18:3)	98.5 ± 1.5a	209.2 ± 9.8b	54.7 ± 2.9c	84.9 ± 7.6a
MGDG (14:0/18:1)	202.8 ± 13.7a	287.6 ± 18.8b	278.6 ± 12.2b	281.2 ± 32.8b
MGDG (16:0/18:2)	289.9 ± 17.0a	440.9 ± 22.5c	318.7 ± 23.4ab	359.1 ± 48.0b
MGDG (16:0/18:1)	271.0 ± 13.7a	477.8 ± 24.1b	467.6 ± 41.3b	495.0 ± 81.8b
DGDG (20:5/18:4)	221.4 ± 17.3a	563.5 ± 90.1b	445.6 ± 69.3b	430.0 ± 39.3b
SQDG (16:4/18:4)	2214.9 ± 56.6a	3814.1 ± 215.6b	1997.8 ± 299.0a	1855.3 ± 76.8a
SQDG (16:3/18:4)	655.8 ± 2.8a	434.5 ± 49.1b	414.3 ± 54.2b	421.9 ± 49.9b
SQDG (18:4/18:4)	2492.1 ± 55.1a	694.1 ± 88.8b	1156.4 ± 160.7c	806.6 ± 12.4b
SQDG (16:2/18:4)	339.9 ± 18.2a	77.6 ± 9.0b	205.8 ± 26.2c	139.0 ± 24.5d
SQDG (18:4/18:3)	865.5 ± 23.9a	194.6 ± 23.5b	462.4 ± 3.1c	248.2 ± 1.7b
SQDG (20:5/16:0)	490.0 ± 18.2a	458.9 ± 18.6b	329.8 ± 73.8a	329.8 ± 1.7a
SQDG (14:0/16:0)	3283.5 ± 416.7a	2112.6 ± 425.3b	2002.7 ± 409.7b	2480.3 ± 108.4ab
SQDG (16:1/14:0)	603.1 ± 18.2a	851.1 ± 116.6b	999.9 ± 103.7b	984.7 ± 39.1b
SQDG (16:2/16:0)	1213.1 ± 167.4a	1727.1 ± 48.7b	1328.3 ± 125.1a	1386.0 ± 72.1a
SQDG (16:0/18:1)	5892.6 ± 425.4a	8877.1 ± 2991.8ab	16,001.6 ± 1688.9c	11,209.4 ± 337.6bc
SQDG (16:1/16:0)	773.0 ± 24.8a	1545.9 ± 133.1b	1807.4 ± 226.6bc	2005.1 ± 171.9c
SQDG (20:1/16:0)	1547.8 ± 19.5	2375.3 ± 611.7	2339.4 ± 302.4	2135.7 ± 4.2
SQDG (16:0/18:3)	771.7 ± 117.4a	1823.2 ± 253.3b	480.9 ± 78.6a	787.9 ± 9.7a
SQDG (16:0/16:0)	9432.0 ± 1410.9a	3384.2 ± 601.2b	8513.4 ± 1127.2a	10,717.8 ± 223.0a
Lyso-SQDG (16:0)	60.7 ± 1.7ab	83.6 ± 7.8c	68.5 ± 4.9b	53.8 ± 0.6a
DGTA (20:5/20:5)	4.0 ± 0.1a	48.5 ± 1.1b	4.9 ± 0.4a	19.9 ± 1.5c
DGTA (20:5/14:0)	27.9 ± 2.0a	292.2 ± 0.0 b	84.3 ± 1.9c	109.0 ± 14.0d
DGTA (20:4/20:5)	74.1 ± 9.8a	511.2 ± 0.4b	128.2 ± 1.1c	155.9 ± 3.3d
DGTA (20:4/14:0)	255.7 ± 30.0a	461.5 ± 9.0b	394.3 ± 23.1c	216.2 ± 14.6a
DGTA (20:5/16:0)	120.4 ± 6.8a	640.6 ± 15.3b	277.6 ± 19.0c	283.6 ± 31.3c
DGTA (20:4/20:4)	582.4 ± 48.8a	803.7 ± 5.7b	1035.8 ± 8.7c	376.8 ± 23.6d
DGTA (20:4/20:3)	91.2 ± 6.8a	149.0 ± 21.1b	217.6 ± 14.6c	51.5 ± 3.1d
DGTA (18:1/14:0)	132.3 ± 2.9a	199.4 ± 1.5b	279.4 ± 12.4c	203.8 ± 15.1b
DGTA (20:4/16:0)	656.0 ± 73.9a	1030.6 ± 25.2b	1227.8 ± 27.7c	452.1 ± 41.5d
DGTA (20:4/18:1)	83.0 ± 7.4a	99.8 ± 1.7b	197.7 ± 5.5c	90.9 ± 4.2ab
DGTA (20:3/16:0)	44.2 ± 0.8a	113.4 ± 0.2b	189.0 ± 13.3c	37.5 ± 1.5a
DGTA (20:4/20:2)	171.7 ± 13.9a	528.0 ± 10.3b	118.4 ± 0.8c	175.2 ± 0.8a
DGTA (18:1/16:0)	127.4 ± 8.2a	154.0 ± 3.1a	342.6 ± 9.4c	197.2 ± 24.6b
DGTA (20:1/20:4)	69.0 ± 3.4a	120.6 ± 2.6b	90.1 ± 2.2c	72.5 ± 4.8a
DGTA (20:2/16:0)	48.6 ± 2.6ab	102.8 ± 0.7c	44.2 ± 2.1b	50.6 ± 2.8a

(Table 1). The contents of DGTA, DGDG, MGDG, and SQDG were different between mature male and female sporophytes, which were higher in mature male sporophytes as compared with mature female sporophytes (Fig. 2). For example, the total content of DGTA in mature male sporophytes was 1.9-fold higher compared with mature female sporophytes.

Changes in lipid composition of receptacles of *S. horneri*

The total contents of DGTA, MGDG, and SQDG in male receptacles were higher than those in female receptacles (Fig. 3). For example, the content of DGTA in male receptacles was 3.0-fold higher than that in female receptacles.

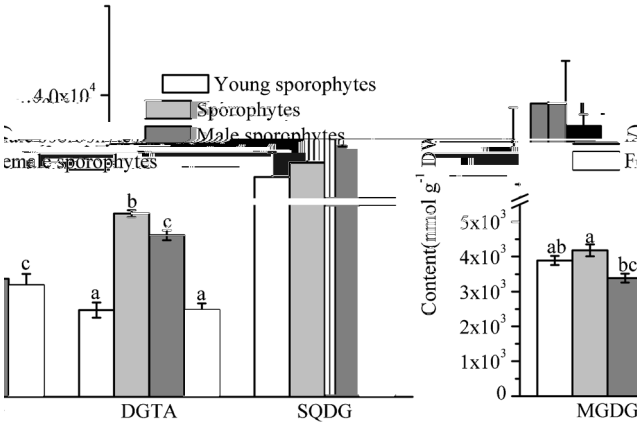

Fig. 2 Content of each lipid class in *S. horneri*. Different letters indicate significant differences (Dunnett's test,` $P < 0.05$) during different growth phases of *S. horneri*

Discussion

Sargassum horneri has a typical chloroplast lipid pattern of a photosynthetic plant with the glycolipids MGDG, DGDG, and SQDG. We found that the content of acidic lipids (SQDG) in *S. horneri* was significantly higher than that in higher plants, which strongly resembled the lipid composition of *Pyropia haitanensis* (Wang et al. 2014). Moreover, this finding was also in agreement with earlier reports in brown algae that have higher SQDG content in this phylum (Khotimchenko 2002; Sanina et al. 2004; Kumari et al. 2013). The reason why the lipid composition in photosynthetic membrane was different from higher plants apparently might be explained by that algae thylakoids are unable to form typical grana stacks of higher plants. This further emphasized the different phylogenetic origins of the algae chloroplast, which originates from a secondary endosymbiosis event (Armbrust et al. 2004). Many algae containing betaine lipids do not contain detectable amount

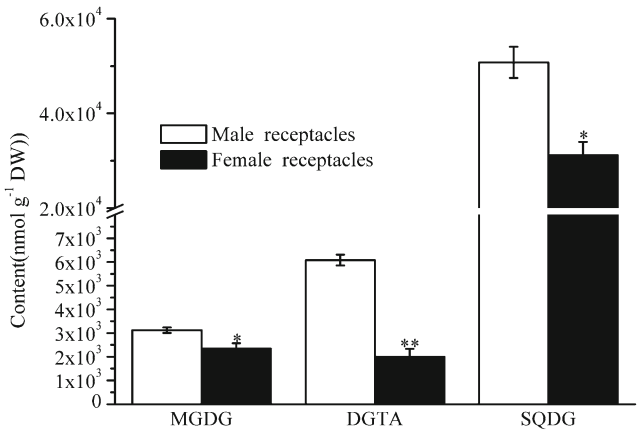

Fig. 3 Content of each lipid class in male receptacles and female receptacles of *S. horneri*. $*P < 0.05$, $**P < 0.01$. The *error bars* represent the standard deviation

of phosphatidylcholine (PC) (Eichenberger and Gribi 1997). The phospholipids (such as PC) were not detected, while the betaine-type lipid DGTA was detected in *S. horneri*. DGTA has structural similarities to PC (Sato and Murata 1991). Therefore, we speculated that the phospholipids were replaced by DGTA in *S. horneri*.

In higher plants, lipids are usually synthesized by two distinct pathways, the prokaryotic and eukaryotic pathways. The lipids synthesized by the prokaryotic pathway have exclusively C16 fatty acids at the *sn*-2 position of glycerol, while the lipids synthesized by the eukaryotic pathway have C18 fatty acids (Xu et al. 2002). MGDG contained high levels of 18:3 and 18:4, of which 18:3 was found at the *sn*-2, and 18:4 was distributed at both *sn*-1 and *sn*-2 positions. The major fatty acids in SQDG were 14:0, 16:0, and 18:1, of which 16:0 was mainly distributed at both *sn*-1 and *sn*-2 position of the glycerol backbone and 18:1 was mainly distributed at the *sn*-2 position. The positional distribution of fatty acids of the individual lipid class of *S. horneri* indicated that MGDG was synthesized by a eukaryotic pathway, because the fatty acids at *sn*-2 position was C18 fatty acids. SQDG had a typical mixed biosynthetic pathway, including both prokaryotic pathway and eyoticic pathway, because the fatty acids at *sn*-2 position include both C16 and C18 fatty acids. The great differences in this synthetic pathway suggested that in the evolutionary process, chloroplasts could gradually use exogenous fatty acids to synthesize their own photosynthe-ic membrane lipids, allowing the host cells to provide liposomal molecules for the synthesis of photosynthetic membrane lipids. Our findings will provide theoretical basis for further study on the ecology, nutrition, and chemical taxonomy of marine algae.

The major fatty acids of MGDG were 18:3, 18:4, 20:5, those of SQDG was 18:1, and those of DGTA was 20:4 in *S. horneri*. Our results showed that algae could be ideal sources for lipids containing highly unsaturated fatty acids compared with terrestrial plants, suggesting that *S. horneri* was a potential source of valuable lipids.

Besides MGDG and DGDG as the membrane components, MGDG and DGDG are also known to fulfill specific molecular functions. They stabilize photosystem subunits (Loll e- al. 2007; Mizusawa and Wada 2012) and bind to the plastid protein import machinery (Schleiff et al. 2003). Jones (2007) has reported that MGDG plays an important role in the photosynthetic membrane, and MGDG may exert an important effect on the gross morphology of the thylakoid membrane. DGDG is exclusively associated with photosynthetic membranes and plays a role in the proper assembly and maintenance of the photosynthetic apparatus (Hartel et al. 1997). Considerable amounts of MGDG and DGDG are thought to exist as "bulk lipids," and their main function is structural lipids (Murata and Siegenthaler 1998). In the present study, the total contents of

MGDG and DGDG were increased initially and then decreased during growth phase of *S. horneri*. This change could be explained by high cell division and photosynthetic membrane development as *S. horneri* grows, and then the degradation of some photosynthetic glycerolipids with the photosynthesis efficiency starting to fall when *S. horneri* became mature. SQDG is bound mainly to the PSII-related complexes among Chl-protein complexes for maintenance of the structural and, inevitably, functional integrity of the PSII complex, but not of the PSI complex (Sato 2004). The total content of SQDG not significantly increased ($P > 0.05$) all the time during growth phase of *S. horneri*. We speculated that SQDG might be at least partially functionally redundant, which might be related to maintenance of anionic charge on the surface of the thylakoid membrane (Mizusawa and Wada 2012). Eichenberger and Gribi (1997) have reported the accumulation of DGTA in non-plastid membranes. It has been reported that the betaine lipid DGTS has the same function as the membrane-associated lipid PC within organisms (Roche and Leblond 2010). As we know DGTA is a structural isomer of DGTS. In the present study, the total content of DGTA was increased initially and then decreased during growth phase of *S. horneri*. This change could be explained by that high cell division and non-plastid membrane development as the growth of *S. horneri*, and when *S. horneri* became mature, the non-plastid membranes started degradation with cytomembrane beginning to decline.

Data also showed that the levels of total lipids, including the MGDG, DGDG, SQDG, and DGTA, in male sporophytes/receptacles were higher than those in female sporophytes/receptacles. It is known that MGDG, DGDG, and SQDG are mainly distributed in chloroplast membrane. Xie et al. (2014) have reported that the PSII photosynthetic capabilities of oogonia continuously weaken with development of *S. horneri*. This result indicated that the photosynthetic capability might be weaker in female sporophytes compared with male sporophytes. As we know, lipids not only play an important role as the structural constituents of most cellular membranes, but also considered as the most effective source of storage energy (Singh et al. 2002). The sperms of *S. horneri* are directly distributed into the sea, while the eggs rely on the transparent mucus to adhere to the surface of receptacles evenly in *S. horneri*. The sperms of *S. horneri* need to swim to the surface of the egg for fertilization. Besides, the number of sperm is also greater than that of egg in *S. horneri*. Therefore, more lipids needed to be synthesized in male sporophytes/000r(d)-3r-2(p)he03omp(ardo)]TJ.0634Tc0-1.25199997TD[(wip)5(r)-309(f55(e11(ma78(le)-395

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