Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 9-13 July 2012
6A Cell and molecular biology of symbiosis

Lipid and fatty acid compositions of *Symbiodinium* strains

Koichiro Awai[1], Ryosuke Matsuoka[2], Yuzo Shioi[2,3]

[1]Division of Global Research Leaders, Shizuoka University, Shizuoka 422-8529, Japan
[2]Faculty of Science, Shizuoka University, Shizuoka 422-8529, Japan
[3]Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan
Corresponding author: dkawai@ipc.shizuoka.ac.jp

Abstract. Many marine invertebrates including corals that live in tropical and subtropical regions form symbioses with dinoflagellates (zooxanthellae). These symbioses are thought to cope with environmental stress by changing the membrane lipid and fatty acid compositions of their zooxanthellae, but details of these compositions have not been reported. To evaluate how the symbiosis withstands stress, we examined the lipid and fatty acid content of the symbiont's membranes. We chose two strains, KB8 and Y106, of *Symbiodinium* which were isolated from a jellyfish *Cassiopea ornata* and a mantle lobe of the giant clam *Tridacna crocea*, respectively. These dinoflagellate strains were cultured in f/2 medium for 21 days at 24ºC. Lipids were extracted from the cells, and separated by two-dimensional thin-layer chromatography. Each lipid was then scraped from the silica plates and co-chromatographed with standard lipids. In both strains, three glycolipids, monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol, were found. These are typical lipids of photosynthetic membranes, indicating that the strains are photosynthetically active. With respect to phospholipids, phosphatidylcholine was the major lipid. This phospholipid is typical of organelles other than the plastids. These dinoflagellates were also found to have long-chain unsaturated fatty acids, e.g., octadecatetraenoic acid, octadecapentaenoic acid, octadecapentaenoic acid, and docosahexaenoic acid. The data presented here provide baseline information to analyze the effects of environmental stress on lipid and fatty acid compositions.

Key words: Membrane lipids, *Symbiodinium*, Polyunsaturated fatty acids, Dinoflagellate, Environmental stress.

Introduction

Marine invertebrates in tropical and subtropical regions, such as jellyfish, shellfish and corals, often contain symbiotic dinoflagellates (zooxanthellae). These symbionts are known to have a pivotal role in adaptation to environmental stresses. Tchernov et al. (2004) suggested that the key determinant of thermal bleaching in zooxanthellate corals is the thylakoid lipid composition of the symbionts. However, they only reported changes in the fatty acid composition of the thylakoid membranes, and exactly which membrane lipids are important remains unknown.

Glycolipids in dinoflagellates have been reasonably well studied. Leblond and his colleagues reported that dinoflagellates contain monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG), typical membrane glycolipids in photosynthetic membranes (Leblond and Chapman 2000; Leblond and Lasiter 2009). These glycolipids are conserved in oxygenic photosynthetic organisms and also in relatives of dinoflagellates such as the Apicomplexa or Chromerida (Botte et al. 2011). Interestingly, the galactolipids of dinoflagellates, MGDG and DGDG possess unusual polyunsaturated fatty acids, namely eicosapentaenoic acid [20:5(n-3)] at the *sn*-1 position and octadecapentaenoic acid [18:5(n-3)] and octadecatetraenoic acid [18:4(n-3)] at the *sn*-2 position (Gray et al. 2009b). Trigalactosyl-diacylglycerol (TGDG) has also been reported in cold-adapted dinoflagellates (Gray et al. 2009a). In contrast, little is known about phospholipids in dinoflagellates.

The effects of low temperature on fatty acid composition have also been analyzed in dinoflagellates (Leblond et al. 2010). It was found that 18:5 fatty acid esterified to the *sn*-2 position of DGDG significantly increased with a decrease in temperature. This observation is similar to the phenomenon found in oxygenic photosynthetic organisms. Cyanobacteria adapt to low temperature by increasing the ratio of polyunsaturated fatty acids (Wada et al. 1990). This is also true for plants (Murata et al. 1992). However, the effect of high temperature on the membrane lipids of dinoflagellates is not yet known.

High temperature is a major stress on the photosynthetic apparatus of symbiotic dinoflagellates. Takahashi et al. (2009) reported that high temperature enhances photodamage of the photosynthetic

machinery by inhibiting its repair. This repair requires *de novo* fatty acid synthesis (Nanjo et al. 2010), which implies a role of membrane lipids in photosynthetic acclimation to high temperature.

Here we report the lipid and fatty acid compositions of *Symbiodinium* strains KB8 and Y106, isolated from a jellyfish *Cassiopea ornata* and a mantle lobe of the giant clam *Tridacna crocea*, respectively. Both *Symbiodinium* strains were found to have similar lipid and fatty acid compositions, with the glycolipids MGDG, DGDG and SQDG being present in the photosynthetic membranes. Phospholipids, such as phosphatidylcholine (PC), were also found. To our knowledge, this is the first detailed analysis of lipid composition in the genus *Symbiodinium*, and these data provide baseline information for analyzing the effects of stress on the membrane lipid compositions of these dinoflagellates.

Materials and Methods

Algae and culture

Symbiodinium strains KB8 (clade A) isolated from a jellyfish *C. ornata* and Y106 (clade A) from the mantle lobe of the giant clam *T. crocea* were obtained from Dr. M. Hidaka (University of the Ryukyus). The dinoflagellates were cultured in f/2 medium (Guillard and Ryther 1962) at 24ºC in the light for 21 days. The algal cultures were continuously illuminated by daylight-fluorescent lamps at an irradiance of 16.2 μmol photons $m^{-2}s^{-1}$.

Lipid analysis

Lipids were extracted according to the method of Bligh and Dyer (1959). Cultured cells were harvested by centrifugation (6,000 *g*, 10 min) and extracted in chloroform/methanol. After two phase extraction, lipids were separated by thin-layer chromatography (TLC) using the solvent systems chloroform: methanol: 28% NH_4OH = 65: 35: 6 (Wada and Murata 1989) for the first dimension, and acetone: toluene: methanol: water: acetic acid = 56: 21: 14: 2: 1 or chloroform: methanol: acetic acid: water = 85: 15: 10: 3.5 for the second dimension. Lipid spots were visualized under ultraviolet light after spraying with primuline (0.01% in 80% acetone). Each lipid was scraped from the plates, and then re-extracted and co-chromatographed by TLC with the standards phosphatidylglycerol (PG), cardiolipin (CL), phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylserine (PS), and PC. For comparison with glycolipids in the photosynthetic membranes, such as MGDG, DGDG and SQDG, lipids of the cyanobacterium, *Synechocystis* sp. PCC 6803, were used. For the standard of a betaine lipid, *Chlamydomonas reinhardtii* was used. The lipid spots were then visualized by spraying anthron reagent or 50% sulfuric acid, followed by baking at 120ºC for 5 min.

For quantification, each lipid was methyl-esterified by methanolic-HCl. Pentadecanoic acid was used as an internal standard. These fatty acid methylesters were then quantified by using a gas-liquid chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a capillary column (BPX90, 0.25 mm x 60 m, SGE Analytical Science, Yokohama, Japan). Temperatures for the injector and detector chambers were set at 250ºC. For the column, temperature was first set at 170ºC and increased at a rate 5ºC min^{-1}. Helium at a flow rate of 1.15 ml min^{-1} was used as the carrier gas. Air at a pressure of 60 kPa and hydrogen at a pressure 50 kPa were used as the flame ionization detector gas.

Results and Discussion

Lipids of *Symbiodinium* strains KB8 and Y106 were extracted and separated by two-dimensional TLC. A typical result is shown in Fig. 1. We detected more than twelve spots on the TLC plates and extracted ten major spots for further analysis. Because the results for *Symbiodinium* KB8 and Y106 are very similar, hereafter we only present the results from *Symbiodinium* strain KB8.

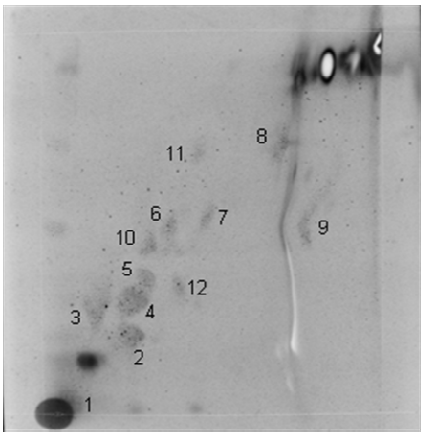

Figure 1: Two-dimensional thin-layer chromatography of membrane lipids in *Symbiodinium* sp. A representative result from strain KB8 is shown. Membrane lipids were visualized with primuline under ultraviolet light. The non-numbered spots are pigments or very minor lipids. Annotations of the numbered spots are described in the text. First dimension (chloroform: methanol: 28% NH_4OH = 65: 35: 6) is vertical and second dimension (chloroform: methanol: acetic acid: water = 85: 15: 10: 3.5) is horizontal.

Isolated lipids were co-chromatographed with standard lipids. TLCs were developed with two solvent systems and according to the R_f values, we determined the lipids to be as follows: 2. DGDG, 3. SQDG, 4. PC, 6. PE, 7. CL, 8. MGDG, 10. PG (Fig.

2). The lipid spots numbered 1, 5, 9, 11, and 12 are unknown. We also extracted lipids from the green alga *C. reinhardtii*, and used these to compare with lipids from *Symbiodinium*. This green alga is known to contain a betaine lipid, diacylglycerol-trimethylhomoserine (DGTS). However, we could not find a lipid that co-migrated with DGTS. Thus, *Symbiodinium* species probably do not have betaine lipids as a membrane constituent. In fact, it is known that most organisms with PC in their membranes do not have betaine lipids. It has been reported that dinoflagellates have sterol lipids (Kokke et al. 1982) and a galactolipid TGDG (Gray et al. 2009a), and the unknown lipids could be one or more of these.

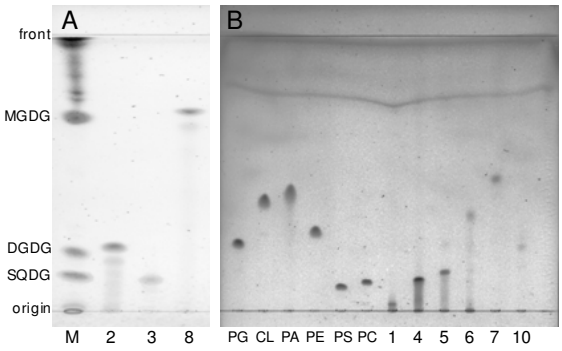

Figure 2: Comparison of extracted lipids and standard lipids. A: Co-chromatogram of glycolipids from *Symbiodinium* strain KB8 and lipids of cyanobacteria as a standard (M). B: Co-chromatogram of standard phospholipids. Both TLC plates were developed by the solvent system chloroform: methanol: acetic acid: water = 85: 15: 10: 3.5. These lipids were also analyzed by another solvent system chloroform: methanol: 28% NH_4OH = 65: 35: 6 and gave the same result. Abbreviations are in the text and numbers of lipids are according to Fig. 1.

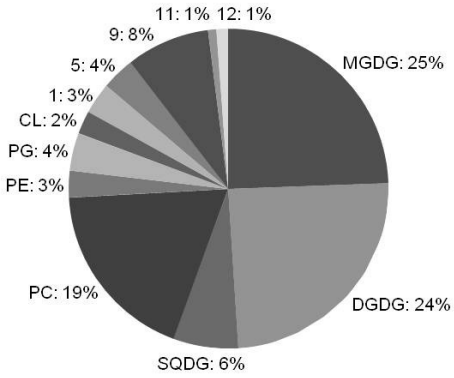

Figure 3: Lipid composition of *Symbiodinium* strain KB8. Abbreviations are defined in the text and numbers of lipids are according to Fig. 1.

Lipid and fatty acid compositions were also analyzed by gas chromatography. As shown in Fig. 3, the major lipids in the *Symbiodinium* cells were glycolipids of the photosynthetic membranes, namely MGDG, DGDG and SQDG. These glycolipids constituted more than half of the total membrane lipids in *Symbiodinium* strain KB8, which indicates that the organism has well developed photosynthetic membranes. With respect to the phospholipid content of the photosynthetic membranes, PG was present in relatively low amounts compared to in other oxygenic photosynthetic organisms. The most abundant phospholipid was PC, which comprised about 20% of total membrane lipids. PE and CL were less abundant. PE is usually found in typical eukaryotic membrane systems, such as the endoplasmic reticulum, Golgi apparatus, mitochondria and plasma membranes. CL is mainly localized in mitochondria in eukaryotic cells. Thus, these organelles/structures are probably not as dominant in *Symbiodinium* cells as in other cell types.

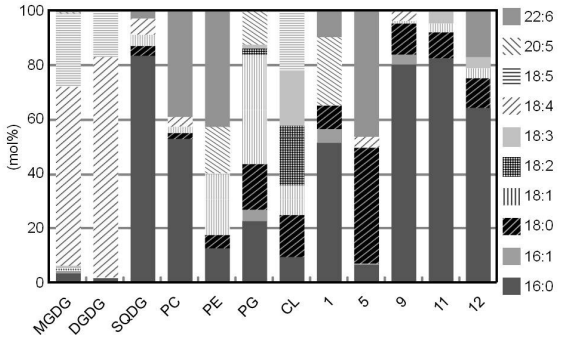

Figure 4: Fatty acid composition of each lipid found in *Symbiodinium* strain KB8. The numbers on the right-hand side indicate numbers of carbons and double bonds (e.g. 22:6 means 22 carbons with 6 double bonds, namely docosahexaenoic acid). The experiments were carried out two times, and the average is shown.

As reported for other dinoflagellates (Leblond et al. 2010), *Symbiodinium* also contained octadecapentaenoic acid [18:5(n-3)] and octadecatetraenoic acid [18:4(n-3)] especially in the galactolipids, MGDG and DGDG (Fig. 4). In MGDG, about 66.1% was 18:4 and 26.9% was 18:5. In DGDG, 81.1% was 18:4 and 16.8% was 18:5. These fatty acids were not major components of the other lipids. In PC and PE, docosahexaenoic acid [22:6(n-3)] was abundant. This polyunsaturated fatty acid comprised 38.8% and 42.8% of PC and PE, respectively. Eicosapentaenoic acid [20:5(n-3)] was detected in PE at 12.4% and PG at 25.1%. The other lipids were mainly comprised of palmitic acid [16:0].

The results shown here suggest that dinoflagellates have a similar lipid composition to other oxygenic photosynthetic organisms. However, their fatty acid composition is quite different. For example, 18:5 fatty acid is very rare and not found in other photosynthetic

organisms. These polyunsaturated fatty acids will be good resources for chemical and/or pharmaceutical applications, and it will be interesting to analyze genes involved in these useful compounds.

Conclusion

In the present study, we analyzed the lipid and fatty acid compositions of *Symbiodinium* strains KB8 and Y106 isolated from *C. ornata* and *T. crocea*, respectively. As far as we know, this is the first detailed report of the lipid and fatty acid compositions of dinoflagellates. The data presented here provide baseline information for analyses of adaptation mechanisms in dinoflagellates in response to environmental stresses.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 19255002) (to YS) and the Japan Science and Technology Agency (to KA).

References

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Botte CY, Yamaryo-Botte Y, Janouskovec J, Rupasinghe T, Keeling PJ, Crellin P, Coppel RL, Marechal E, McConville MJ, McFadden GI (2011) Identification of plant-like galactolipids in *Chromera velia*, a photosynthetic relative of malaria parasites. J Biol Chem 286:29893-29903

Gray CG, Lasiter AD, Leblond JD (2009a) Mono- and digalactosyldiacylglycerol composition of dinoflagellates. III. Four cold-adapted, peridinin-containing taxa and the presence of trigalactosyldiacylglycerol as an additional glycolipid. Eur J Phycol 44:439-445

Gray CG, Lasiter AD, Li C, Leblond JD (2009b) Mono- and digalactosyldiacylglycerol composition of dinoflagellates. I. Peridinin-containing taxa. Eur J Phycol 44:191-197

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. Can J Microbiol 8:229-239

Kokke WCMC, Fenical W, Djerassi C (1982) Sterols of the cultured dinoflagellate *Pyrocystis lunula*. Steroids 40:307-318

Leblond JD, Chapman PJ (2000) Lipid class distribution of highly unsaturated long chain fatty acids in marine dinoflagellates. J Phycol 36:1103-1108

Leblond JD, Lasiter AD (2009) Mono- and digalactosyldiacylglycerol composition of dinoflagellates. II. *Lepidodinium chlorophorum*, *Karenia brevis*, and *Kryptoperidinium foliaceum*, three dinoflagellates with aberrant plastids. Eur J Phycol 44:199-205

Leblond JD, Dahmen JL, Evens TJ (2010) Mono- and digalactosyldiacylglycerol composition of dinoflagellates. IV. Temperature-induced modulation of fatty acid regiochemistry as observed by electrospray ionization/mass spectrometry. Eur J Phycol 45:13-18

Murata N, Ishizaki-Nishizawa O, Higashi S, Hayashi H, Tasaka Y, Nishida I (1992) Genetically engineered alteration in the chilling sensitivity of plants. Nature 356:710-713

Nanjo Y, Mizusawa N, Wada H, Slabas AR, Hayashi H, Nishiyama Y (2010) Synthesis of fatty acids de novo is required for photosynthetic acclimation of *Synechocystis* sp. PCC 6803 to high temperature. Biochim Biophys Acta 1797:1483-1490

Takahashi S, Whitney SM, Badger MR (2009) Different thermal sensitivity of the repair of photodamaged photosynthetic machinery in cultured *Symbiodinium* species. Proc Natl Acad Sci USA 106:3237-3242

Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, Haggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc Natl Acad Sci USA 101:13531-13535

Wada H, Murata N (1989) *Synechocystis* PCC6803 mutants defective in desaturation of fatty acids. Plant Cell Physiol 30:971-978

Wada H, Gombos Z, Murata N (1990) Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. Nature 347:200-203