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Fatty acid variations in symbiotic dinoflagellates from Okinawan corals

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Abstract

The fatty acid composition of polar lipids and triacylglycerols was determined in different morphophysiological types of symbiotic dinoflagellates (SD) isolated from the hydrocoral *Millepora intricata* and the scleractinian corals *Pocillopora damicornis*, *Seriatopora caliendrum*, *Seriatopora hystrix* and *Stylophora pistillata* from a fringing reef of Sesoko Island, Okinawa, Japan. The distribution of the fatty acids among the morphophysiologically distinct types of SD reported in these corals makes it possible to readily distinguish one type of SD from the other. Moreover, differences were found both in polar lipids and triacylglycerols. The polar lipids of SD from *M. intricata* showed a very distinctive fatty acid profile. A combination of large proportions of 18:4 (n-3), 18:5 (n-3), 22:5 (n-6), and 22:6 (n-3) and negligible amounts of 20:4 (n-6), and 20:5 (n-3) in SD from *M. intricata* was particularly noteworthy. The fatty acid profiles of SD from *P. damicornis* and SD isolated from *S. caliendrum* and *S. hystrix* differed in the proportion of 18:4 (n-3) and 22:6 (n-3). It is suggested that fatty acids might provide useful information on possible taxonomic differences among symbiotic dinoflagellates. It is assumed that biochemical differences can reflect the genetic diversity of the morphophysiological types of SD associated with several species of hermatypic corals from this region.

Keywords: Dinophyceae; Symbiotic dinoflagellates; Zooxanthellae; Hermatypic corals; Taxonomy; Fatty acids

1. Introduction

Investigations of the symbiotic dinoflagellates (SD) associated with hermatypic corals have shown that they comprise a group of diverse taxa (Rowan, 1998). To date, dinoflagellates that exhibit a symbiotic mode of life are represented by eight genera in four orders within the division Dinophyta. A combination of morphological, ultrastructural, physiological, biochemical and karyotypic characteristics is useful in demonstrating genotypic variations of SD from marine invertebrates, including hermatypic corals (Trench, 1997).

Three morphophysiological types of symbiotic dinoflagellates from Okinawan hermatypic corals were identified: "L" (large), "B" (brown) and "G" (green) (Titlyanov et al., 2001). Colonies of the hydrocoral *Millepora intricata* (Milne Edwards, 1857) hosted symbionts of type L only; among scleractinian corals, only *Pocillopora damicornis* (Linnaeus, 1758) contained type B; type G was found only in *Seriatopora caliendrum* (Ehrenberg, 1834) and *S. hystrix* (Dana, 1846), and both type B and G occurred in *Stylophora pistillata* (Esper, 1797). The different types of SD significantly differed in cell size and shape and structural elements in coccoid state *in hospite*. SD of the three types also differed in photosynthetic capacities, primary production, pigment accumulation, and maximum rates of cell division and degradation (Titlyanov et al., 2001). Nevertheless, even significant morphophysiological differences are still insufficient to identify these SD types as population of different species. A further study was needed to determine the level of taxonomic differences between these morphophysiological types of SD.

Microalgae are known to have different fatty acid compositions depending on their taxonomic position (Ackman et al., 1968; Volkman et al., 1989; Dunstan et al., 1992; Zhukova and Aizdaicher, 1995). The fatty acid analysis of algae can be used as a guide in algal taxonomy (Maruyama et al., 1986; Gladu et al., 1995), in screening water (Skerratt et al., 1995; Napolitano et

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al., 1997) and for the determination of the origin of organic matter pools (Fileman et al., 1998). Although fatty acid composition shows distinctive and reproducible differences between microalgal classes, it can only rarely be used to distinguish between genera or species within an algal class. Variability of fatty acid compositions of different species belonging to the same class (Volkman et al., 1991) and of strains of the *Isochrysis* species (Lopez Alonso et al., 1992) has been demonstrated. Evidence for the importance of the genome in determining fatty acid content in *Isochrysis galbana* has been reported and the majority of the fatty acids has high heritability in collection and strains of *I. galbana* (Lopez Alonso et al., 1994). Intraspecific genetic variation was also reported in the fatty acids of *Sceletonema costatum* (Shaw et al., 1989).

There are few works about fatty acids of symbiotic zooxanthellae (Bishop and Kenrik, 1980; Miralles et al., 1989). The fatty acid composition of zooxanthellae isolated from different species of corals, clams and a foraminifera was found to vary according to the host (Bishop and Kenrick, 1980). The authors suggested that lipid analysis might be a rapid and reliable mean of identifying differences between the great numbers of zooxanthellae, which infect marine invertebrates.

Data on fatty acid composition might thus provide valuable chemotaxonomic markers for differentiating the morphological types of SD from hermatypic corals. Analysis of the fatty acids of the SD isolated from the hydrocoral and four species of scleractinian corals preadapted to identical environmental conditions was performed in the present study.

2. Results

Table 1 shows the fatty acid composition of polar lipids of symbiotic dinoflagellates (SD) isolated from five species of hermatypic corals acclimated to 30% of photosynthetic active radiation. The fatty acid composition of SD was found to vary according to the coral species, but in general it was typical for dinoflagellates. Among the saturated acids, 16:0 prevailed (12.8–18.6%). The polar lipids of SD were characterized by high concentrations of polyunsaturated fatty acids (60.1–72.1%), in particular n-3 series. Among these, 18:4 (n-3) and 22:6 (n-3) were predominant in all species. SD isolated from *M. intricata* exhibited a very distinctive fatty acid profile, with the following five major fatty acids: 16:0, 18:4 (n-3), 18:5 (n-3), 22:5 (n-6), and 22:6 (n-3) (Table 1). ANOVA analysis showed significant differences ($P<0.01$) in these fatty acids for *M. intricata* and scleractinian corals. These components accounted for approximately 75% of the total fatty acids. The fatty acid 18:4 (n-3) constituted 26.2% of the total fatty acids. In contrast to SD from four species of scleractinian corals, SD from *M. intricata* was rich in 18:5 (n-3) and 22:5 (n-6), whereas 20:5 (n-3) and 20:4 (n-6) were detected in trace amounts. SD from *P. damicornis* and from two species of *Seriatopora* showed fairly similar fatty acid profiles with five predominant components: 16:0, 18:4 (n-3), 20:4 (n-6), 20:5 (n-3), and 22:6 (n-3). Of these, 18:4 (n-3) was present in higher amounts in SD from *P. damicornis* than in SD from *Seriatopora* species, whereas the reverse was true for 22:6 (n-3) ($P<0.05$). Although SD from *P. damicornis* contained higher concentration of 18:4 (n-3) than SD from *Seriatopora* species; however, its content was lower compared to that of SD from *M. intricata*. Polar lipids of SD of scleractinian corals were poor in 18:5 (n-3) (about 1%), but they exhibited high concentrations of the fatty acids of n-6 series, 18:3 (n-6), and 20:4 (n-6). The fatty acid pattern of SD isolated from *S. pistillata* was largely similar to that of *P. damicornis* and *Seriatopora* species. SD from *S. pistillata* were richer in 18:4(n-3) (17.2%) and poorer in 22:6(n-3) (11.2%) than SD from *Seriatopora* species. Percentage distribution of SD from *S. pistillata* resembled that of SD from *P. damicornis*.

Unlike the membrane polar lipids, the triacylglycerols profiles of SD were simple (Table 2). Marked differences in the level of some fatty acid components were detected, especially for 16:0, 16:1 (n-7), 18:0 and 22:6 (n-3).

Table 1
Fatty acid composition of polar lipids of symbiotic dinoflagellates from hermatypic corals (% of total fatty acids, values more than 0.5% are given)

Coral species	*Millepora intricata*	*Pocillopora damicornis*	*Seriatopora caliendrum*	*Seriatopora hystrix*	*Stylophora pistillata*
SD types	Type L	Type B	Type G	Type G	Types B and G
12:0	0.5	0.4	0.3	0.4	0.1
14:0	2.4	2.9	2.7	2.3	2.5
14:1	1.0	0.5	1.0	0.6	0.2
16:0	12.8	16.7	16.6	17.7	18.6
16:1(n-7)	2.1	2.8	4.3	2.9	2.9
17:0	0.2	0.3	0.2	0.2	0.3
18:0	5.1	7.9	6.9	7.6	6.9
18:1(n-9)	1.8	1.9	2.5	1.8	2.7
18:1(n-7)	0.6	1.0	0.8	1.0	0.9
18:2(n-6)	0.6	1.6	1.7	1.4	1.2
18:3(n-6)	1.7	2.9	5.2	4.3	1.5
18:3(n-3)	–	0.5	0.5	0.3	0.4
18:4(n-3)	26.2	17.5	10.3	10.0	17.2
18:5(n-3)	8.7	1.3	0.6	0.7	0.8
20:1	0.7	0.7	0.7	1.8	0.9
20:2(n-6)	0.4	1.1	0.8	0.8	0.8
20:3(n-6)	0.4	0.6	0.3	0.5	0.4
20:4(n-6)	0.1	7.5	8.6	11.3	7.5
20:5(n-3)	0.5	11.2	14.8	10.3	16.1
22:0	–	0.3	0.3	–	0.2
22:4(n-6)	4.9	4.6	3.0	3.5	2.1
22:5(n-6)	10.3	–	–	–	–
22:5(n-3)	0.5	1.4	0.9	1.1	2.1
22:6(n-3)	17.8	10.6	16.4	15.9	11.2

Values represent the mean of the three replicates, variability is not reported but was under 5%.

Table 2
Fatty acid composition of triacylglycerols of symbiotic dinoflagellates from hermatypic corals (% of the total fatty acids, values more than 0.5% are given)

Coral species	*Millepora intricata*	*Pocillopora damicornis*	*Seriatopora caliendrum*	*Seriatopora hystrix*	*Stylophora pistillata*
SD types	Type L	Type B	Type G	Type G	Types B and G
12:0	0.2	0.2	0.8	0.2	0.6
14:0	2.9	4.6	8.2	8.0	5.1
16:0	26.0	42.3	31.4	32.0	37.2
16:1(n-7)	1.0	5.8	11.9	12.3	6.2
17:0	0.4	–	0.5	0.2	0.2
18:0	20.9	5.0	4.8	4.4	4.3
18:1(n-9)	4.2	8.9	8.7	6.1	14.1
18:1(n-7)	0.4	1.1	1.3	1.3	2.0
18:2(n-6)	1.2	2.1	3.1	2.0	0.9
18:3(n-6)	1.8	2.5	0.5	0.3	1.1
18:3(n-3)	–	0.2	1.0	0.2	0.2
18:4(n-3)	–	0.2	–	–	0.1
18:5(n-3)	0.1	–	–	0.3	–
20:1	–	–	–	0.3	–
20:2(n-6)	0.3	0.1	–	0.6	–
20:3(n-6)	–	0.1	0.5	–	–
20:4(n-6)	–	0.4	1.3	0.6	0.5
20:5(n-3)	–	2.5	4.0	4.5	3.4
22:0	–	0.1	–	0.1	–
22:4(n-6)	1.0	0.5	0.5	0.4	0.4
22:5(n-3)	–	0.7	0.5	1.0	1.4
22:6(n-3)	39.2	21.8	19.3	23.3	21.2

Values represent the mean of the three replicates, variability is not reported but was under 5%.

The fatty acid composition of triacylglycerols of SD from *M. intricata* differed significantly from that of other SD investigated ($P < 0.05$). The major fatty acid of SD from the hydrocoral *M. intricata* was 22:6 (n-3), which accounted for 39% of the total fatty acids and 16:0 (26%), whereas the reverse ratio occurred in triacylglycerols of SD from scleractinian corals (31.4–42.3% versus 19.3–23.3%). The 18:0 fatty acid constituted more than 20% of the total fatty acids in triacylglycerols of SD from *M. intricata*, but it made up only about 5% in triacylglycerols of SD from scleractinian corals. The content of 16:1(n-7) in triacylglycerols of SD from *M. intricata* was about 1%, whereas in the same lipid class of SD from scleractinian corals its proportion ranged from 6 to 12%. Some differences were found in fatty acid composition of triacylglycerols of SD from *P. damicornis* and *Seriatopora* species. SD of *P. damicornis* were richer in 16:0 than *Seriatopora* species (42 versus 31%) but had lower levels of 16:1(n-7) (6 versus 12%). The fatty acid composition of triacylglycerols of SD from *S. pistillata* was similar to that of *P. damicornis* (Table 2).

3. Discussion

Different morphophysiological types of symbiotic dinoflagellates (Titlyanov et al., 2001), in general, exhibited the characteristic features of the fatty acid composition specific for dinoflagellates and zooxanthellae. The high proportions of 18:4 (n-3) and 22:6 (n-3) in polar lipids, the latter acid is rare in other microalgae, were in agreement with the previous analyses for dinoflagellates (Joseph, 1975) and zooxanthellae (Bishop and Kenrick, 1980). However, the distribution of the fatty acids among the morphophysiological types of SD makes it possible to readily distinguish one type from the other. The polar lipids of SD from *M. intricata* (type L according Titlyanov et al., 2001) showed a very peculiar profile with the following five major fatty acids: 16:0, 18:4 (n-3), 18:5 (n-3), 22:5 (n-6), and 22:6 (n-3). The main feature was the negligible amounts of the fatty acids 20:4 (n-6) and 20:5 (n-3), which were abundant in other types of SD. A combination of large proportions of 18:4 (n-3), 22:5 (n-6), 22:6 (n-3) and negligible amounts of 20:4 (n-6) and 20:5 (n-3) detected in SD from *M. intricata* (type L) was particularly impressive. The acid 20:5 (n-3) is usually abundant in dinoflagellates (Joseph, 1975). Trace amounts of 20:5 (n-3) were found in some species, for example, *Gymnodinium* cf.*nagasakiense* (Parrish et al., 1994), but the distribution of other fatty acids was typical of dinoflagellates. The other main difference was the large amount of 18:5 (n-3) in polar lipids of symbionts of type L (from *M. intricata*). This acid was first identified in dinoflagellates (Joseph, 1975), and it has been proposed as a marker for this class of microalgae. It was subsequently found also in prymnesiophyte and raphidophyte algae (Dunstan et al.; 1992, Bell et al., 1997). A remarkable component was 22:5 (n-6). It amounted to 10% of the total fatty acids in SD from *M. intricata*, whereas it was absent in other types of SD. The distribution of fatty acids in type L was very similar to that of zooxanthellae isolated from clams (Bishop and Kenrick, 1980). The morphophysiological type L symbionts were found exclusively in the hydrocoral *M. intricata* (Titlyanov et al., 2001). SD of the type L have not been described in the literature. Cells *in hospite* were 13–18 μm in diameter (Titlyanov et al., 2001), considerably larger than those of the alga *Gloeodinium viscum* (11–13 μm) associated with *Millepora dichotoma* (Banaszak et al., 1993).

The fatty acid profiles of polar lipids of SD types B (from *P. damicornis*) and G (from *S. caliendrum* and *S. hystrix*) were rather similar, but the percentage distribution of some fatty acid components differed markedly between these types. The major variations were in the levels of 18:4 (n-3) and 22:6 (n-3). Type B differed from type G by the reversed proportions of these fatty acids. Our data on fatty acids of SD type B from *P. damicornis* are in agreement with the results of Bishop and Kenrick (1980) for this species. SD type G from *S. caliendrum* and *S. hystrix* showed a similar fatty acid composition. As regards symbionts from *S. pistillata*

(types B and G), their fatty acid composition was more similar to type B than to type G, suggesting the predominance of algae of the type B. This is in consistent with morphological data (Titlyanov et al., 2001).

The results of lipid analysis clearly show that SD of different morphophysiological types have different fatty acid compositions. Moreover, differences were found not only between fatty acids of polar lipids, which are structural components of cell membranes, but also between fatty acids of triacylglycerols, which are storage compounds. It has been suggested that the fatty acid variations between strains of *Isochrysis* species are related to genetic differences (Volkman et al., 1989). It has been shown that collection strains and isolates of *Isochrysis galbana* differ in their ability to produce fatty acids and that these differences are to a large extent genetically determined (Lopez Alonso et al., 1992). A significant genetic component in the variance of some of the major fatty acids of *Phaeodactilum tricornutum* strains has been determined (Lopez Alonso et al., 1994). Since SD were isolated from coral colonies that were exposed to identical environmental conditions, the differences in the fatty acid composition among SD types can reflect the genetic diversity of the morphophysiological types of SD associated with several species of hermatypic corals from this region. SD from *M. intricata* (type L according to Titlyanov et al., 2001) is clearly distinct from the SD of scleractinian corals (types B and G) by the phylogenetic position. Based on these data we suggest that chemotaxonomic differences, particularly regarding fatty acids, may be useful in differentiating symbiotic dinoflagellates from marine invertebrates.

4. Experimental

4.1. Isolation of symbiotic dinoflagellates

The coral colonies of *Stylophora pistillata*, *Seriatopora caliendrum*, *S. hystrix*, *Pocillopora damicornis*, and colonial hydroid *Millepora intricata* were collected from a fringing reef at Sesoko Station, the Tropical Biosphere Research Center, University of Ryukyus, Okinawa, Japan. Medium-size samples were collected at a depth of 2 m. Samples were stored in 12 m^3 outdoor aquariums for 150 days at 30% of photosynthetic active radiation. Conditions of maintenance were described in details elsewhere (Titlyanov et al., 2001). Symbiotic dinoflagellates were isolated from three different colonies of each coral species. SD liberated from coral tissue by the Water-Pik method (Johannes and Wiebe, 1970) were cleared of coral tissue remnants by sedimentation and double centrifugation, followed by washing with filtered seawater. SD from scleractinian corals were collected on a 5 μm Isopore membrane filters (TMTP 5 μm). SD from *M. intricata* were concentrated on a 12 μm Isopore membrane filters (TMTP 12 μm). The purity of the SD fractions was examined using light microscopy (400×). Filters with SD were placed in $CHCl_3$–MeOH (1:1, v/v) and stored at −20 °C until analysis. Triplicate samples for each coral species were obtained for lipid extraction.

4.2. Fatty acid analyses

Lipids were extracted with $CHCl_3$–MeOH (1:2) (Bligh and Dyer, 1959). The combined extracts from each sample were partitioned with $CHCl_3$ and H_2O. Lipids were recovered in the lower $CHCl_3$ phase, the solvents were removed under vacuum, and the lipid extracts were stored in $CHCl_3$. Preparative separation of lipid classes was performed by silica gel TLC using hexane–Et_2O–HOAc (80:20:1) as development solvent. The zones of the polar lipids and triacylglycerols were immediately scraped and eluted with $CHCl_3$–MeOH (1:1). Fatty acids were converted to Me esters using 1% Na in MeOH, followed by 5% HCl in MeOH (Carreau and Dubacq, 1978) and purified by silica gel TLC using benzene. The resulting FAMEs were analyzed by FID-GC (Shimadzu GC-9A) with fused silica capillary column (30 m×0.25 mm), coated with Supelcowax 10, column temperature was 210 °C. He was used as a carrier gas, split 1:30. Individual peaks of FAMEs were identified by comparing R_t data with those of authentic standards and using ECL measurements. Additionally, $AgNO_3$–TLC was used for the identification of unsaturated fatty acids. The fatty acid composition of the samples was expressed as a percentage of the total fatty acids. Data reported are averages of the replicates for each species. The differences among means were analyzed by one-way ANOVA. Differences are reported as significant when $P<0.05$. Statistical analysis was conducted on the main fatty acids.

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