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Diversity of fatty acid composition of symbiotic dinoflagellates in corals: Evidence for the transfer of host PUFAs to the symbionts

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ABSTRACT

High diversity of fatty acid (FA) composition of endosymbiotic dinoflagellates of the *Symbiodinium* group (zooxanthellae) isolated from different cnidarian groups has been found. To explain this diversity, FA composition of the total lipids of pure symbiont fractions (SF) and host cell tissue fractions (HF) isolated from one hydrocoral, two soft coral, and seven hard coral species inhabiting the shallow waters of the South China Sea (Vietnam) were compared. *Symbiodinium* phylogenetic clade designation for each SF was also determined, however, the relationship between the clade designation and FA composition of *Symbiodinium* was not found. The profiles of marker polyunsaturated FAs (PUFAs) of symbionts (18:4n-3, 18:5n-3, 20:5n-3) did not depend on taxonomic designation of the host and reflected only a specimen-specific diversity of the SF lipids. Several FAs such as 20:0, C_{24} PUFAs, 22:5n-6, and 18:2n-7 concentrated in HF lipids but were also found in SF lipids. For ten cnidarian species studied, the principal components analysis of total FAs (27 variables) of the symbiotic fractions was performed. The clear division of the symbiotic dinoflagellates according to the host systematic identity was found on a subclass level. This division was mainly caused by the FAs specific for the host lipids of each cnidarian subclasses such as hard corals, soft corals, and hydrocorals. Thus, the coral hosts affect the FA profile of their symbionts and cause the diversity of FA composition of *Symbiodinium*. The transfer of FAs from the coral host to their symbiotic dinoflagellates and modulation of PUFA biosynthesis in symbionts by the host are considered as possible reasons of the diversity studied.

Introduction

Many coral species acquire photosynthetically-fixed carbon by forming symbioses with endocellular dinoflagellates of the *Symbiodinium* group (zooxanthellae) (Venn et al., 2008). The phototrophic supply is the most important carbon source for zooxanthellate corals; in some corals more than 90% of photosynthate may be released by symbiotic dinoflagellates to its host cell (Muscatine et al., 1981; Sorokin, 1993). The loss of zooxanthellae and their photosynthetic pigment associated (named coral bleaching) results in large-scale coral mortality and degradation of coral reef communities (e.g., Brown, 1997).

Zooxanthellae obtain from the host cell the carbon dioxide, water for the photosynthetic reaction, and all substrates for synthesis of cellular constituents. Zooxanthellae release various low molecular weight compounds, including glycerol, organic acids, glucose, and amino acids transferred to the coral host tissues (Grant et al., 1997). In corals, lipids are the main long-term source of stored energy (Harland et al., 1993) and are involved in a majority of biochemical and physiological processes (Ref. Rodrigues et al., 2008). A possible transfer of lipids from symbionts to the host was studied in hard corals. Patton et al. (1983) assumed that a part of the photosynthetically fixed carbon was immediately synthesized into lipids translocated from zooxanthellae to the host. Treignier et al. (2009) suggested that in *Turbinaria reniformis* some lipids were transferred from the symbionts to the host. Zooxanthellae influence the ratio between polar and neutral lipids in the scleractinian *Pachyseris speciosa* and *Seriatopora hystrix* and may control the energetic status of the host (Cooper et al., 2011). The direct translocation of lipids of zooxanthellae to the coral host has not well documented, and a possible mechanism of this process is under discussion (Muscatine et al., 1994; Luo et al., 2009; Dunn et al., 2012).

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The primary characteristic of lipids is the composition of their fatty acids (FAs) included in lipid molecules as acyl groups. The FA composition of total lipids of whole colonies of numerous zooxanthellate coral species has been described (Imbs et al., 2010a; Pham and Imbs, 2012), but the data on FA composition of zooxanthellae freshly isolated from a coral colony are very limited (Bishop and Kenrick, 1980; Al-Moghrabi et al., 1995; Zhukova and Titlyanov, 2003). Only Papina et al. (2003) and Treignier et al. (2008, 2009) have determined the FA composition both of zooxanthellae and of the host of the same hard coral colony. Among soft corals, FA composition of zooxanthellae and the host have been described in *Sinularia* sp. only (Imbs et al., 2010c).

Pathways of PUFA biosynthesis in animals and plants are different. Contrary to plants, double bonds cannot be inserted beyond the Δ9 position into FA molecules of animal tissues, and animals cannot synthesize 18:2n-6 and 18:3n-3 (Bachok et al., 2006). Therefore, zooxanthellae (plants) and the coral host (animals) contain the different PUFAs, which can serve as the markers of either symbiont lipids or host lipids. These specific FA markers can be applied to confirm the exchange of PUFAs between symbionts and the host.

C_{16} PUFAs, 18:3n-6, 18:4n-3, and 18:5n-3 mainly synthesized in zooxanthellae were suggested as the markers of coral symbionts (Bishop and Kenrick, 1980; Papina et al., 2003; Imbs et al., 2010b). For some coral species, the presence of these PUFA markers in the host lipids have been regarded as the confirmation of PUFA transfer from zooxanthellae to the host (Papina et al., 2003; Treignier et al., 2008; Imbs et al., 2010b). Several PUFAs are mainly synthesized in host coral tissue and can be used as the host markers (Latyshev et al., 1991; Imbs et al., 2007b, 2009, 2010a,b; Pham and Imbs, 2012). The host FA markers were not applied to study lipid transfer from the host to their symbiotic dinoflagellates. It is unknown if coral host influence biosynthesis of FA in their symbionts or not.

In our study, FA composition diversity of the symbiotic dinoflagellates of cnidarians was examined. Possible causes of this diversity regarding *Symbiodinium* phylogenetic clades and PUFA transfer in coral symbiont-host association were suggested.

Results

Ten symbiotic cnidarians (one hydrocoral, two soft coral and seven hard coral species) inhabiting the shallow waters of Nha Trang Bay (Vietnam) were investigated (Table 1). Pure symbiont fractions (SF) and host fractions (HF) were isolated from each cnidarian species. Comparative analysis of the clade designation of symbionts (Table 1) and FA composition of total lipids in HF and SF was performed (Table S1).

Symbiodinium of clade D/D1 were found in *Montipora digitata* and *Pocillopora damicornis*. Other cnidarian species contained zooxanthellae of clade C. The diversity of phylogenetic clades (C15, C16, and C17) was observed within individual colonies of *Porites cylindrica*. The relationship between the clade designation of zooxanthellae and their FA composition was not found. For example, common clade C71a of the symbionts were isolated from *Sinularia* cf. *capitalis*, *S. polydactila*, and *Acropora intermedia* (Table 1), but FAs of SF from *Sinularia* contained specific acids 16:3n-4, 16:4n-1, 24:5n-6 and 24:6n-3, which were absent in SF from *A. intermedia* (Table S1). The contents of common FA in SF from *Sinularia* and *Acropora* also had significant differences (Table 2). Similarly, the differences in FA percentages were found following the comparison of clade D/D1a isolated from *M. digitata* and *P. damicornis* (Table 2).

In the lipids of all SF obtained, the main PUFAs were 18:4n-3, 20:4n-6, 20:5n-3, and 22:6n-3 (Table S1). Rare 18:5n-3 was also detected. SF from the hard corals were rich in 18:3n-6. Acids 16:3n-4 and 16:4n-1 were characteristic for SF from the soft corals. Acids 18:3n-6, 18:4n-3, 18:5n-3, 16:3n-4, and 16:4n-1 were concentrated in SF lipids, but small amount of these acids were found in HF lipids (Table S1 and Fig. 1). The percentage of 20:5n-3 and 22:6n-3 in SF lipids was higher than that in HF lipids. The percentage of 22:4n-6 was mostly higher in HF from the hard corals. Acids characteristic of HF lipids (24:5n-6 and 24:6n-3) were detected in SF lipids of the soft corals.

The hydrocoral SF contained high percentage of 20:0, 22:5n-6, and 22:6n-3 in comparison with the coral SF. The specific PUFA of *Millepora* (22:5n-6) were found both in HF and in SF lipids (Table S1 and Fig. 1).

The content of marker PUFAs and total FAs in SF lipids was compared according to the host taxonomic groups. The relative percentages of *Symbiodinium* marker PUFAs (18:4n-3, 18:5n-3, and 20:5n-3) in SF differed significantly (Table 3). To show visually this difference, *Symbiodinium* marker FAs (16:1n-9, 18:4n-3, 18:5n-3, and 20:5n-3) were used as variables for principal component analysis (PCA). All SF formed a diffusive group into the plot representation (Fig. 2A). Loading factors for each variable are showed in Table S2. The distribution of SF was specimen-specific, but SF isolated from hard corals, soft corals or hydrocorals did not form separate groups with respect to *Symbiodinium* marker PUFAs.

Another result was obtained when total FAs of SF were used as variables. PCA demonstrated how the symbiont samples were similar with respect to their FA composition (Fig. 2B). The PCA illustrated the grouping of FA profiles of SF according to the host systematic identity. The FA composition of SF isolated from the hard corals differed significantly from that of SF obtained from the soft corals or hydrocorals. Two principal component axes described 53.9% of the variation between the samples. Loading factors for each variable are showed in Table S2. C_{24} PUFAs, C_{16} PUFAs, 18:2n-7, 16:0 (PC1 axis), as well as 22:5n-6 and 20:0 (PC2 axis), were responsible for the distinctions of samples in component space (factor loading of variables was more than 0.8). The influence of 18:3n-6, 20:5n-3, 22:4n-6, and 22:6-3 was lower.

C_{24} PUFAs were present in SF and HF of soft corals only (Table S1). In the soft and hard corals, 22:5n-6 was present in negligible quantity (0–0.3% of total FA content; Table S1). C_{16} PUFAs

Table 1
List of the host species sampled their identification and clade designation of the symbiont isolated from the host species.

Systematic affiliation of cnidarians	Host species sampled	Clade designation of symbiont
Hydrozoa (hydrocorals)	*Millepora platyphylla* (Hemprich & Ehrenberg, 1834)	C66a
Anthozoa: Octocorallia (soft corals)	*Sinularia* cf. *capitalis* (Pratt, 1903)	C71a
	Sinularia polydactila (Ehrenberg, 1834)	C71a
Anthozoa: Hexacorallia (hard corals)	*Acropora intermedia* (Brook, 1891)	C71a
	Acropora muricata (Linnaeus, 1758)	C1f/C27
	Montipora digitata (Dana, 1846)	D/D1a
	Montipora foliosa (Pallas, 1766)	C60
	Pavona decussata (Dana, 1846)	C72, C1b
	Pocillopora damicornis (Linnaeus, 1758)	D/D1a
	Porites cylindrica (Dana, 1846)	C15, C16, C17