Vietnam Journal of Marine Science and Technology 2024, 24(3) 289–296

Vietnam Academy of Science and Technology

Vietnam Journal of Marine Science and Technology

journal homepage: vjs.ac.vn/index.php/jmst

Diversity of fatty acids in different coral species collected in the coastal sea of Nha Trang, Khanh Hoa

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Received: 9 April 2024; Accepted: 20 May 2024

ABSTRACT

The total of 15 coral samples belonging to soft coral species *Sinularia flexibilis*, hard coral *Acropora* sp., and the hydrocoral *Millepora platypylla*, were collected in the coastal sea of Nha Trang, Khanh Hoa, Vietnam. In these samples, 39 fatty acids were identified in which *Sinularia flexibilis* sample identified 26 fatty acids, *Acropora* sp. identified 27 fatty acids, and *Millepora platyphylla* has the most diverse fatty acid composition with 35 fatty acids. In the coral samples of *S. flexibilis*, the difference between the ratio of total saturated fatty acids and the polyunsaturated fatty acids was much smaller than that of the other two species. The n-3/n-6 ratio is the highest among *Millepora* species, up to 4.45; in *S. flexibilis* and *Acropora*, this ratio can be lower than 1. Fatty acids 16:0 and 18:0 are the two main fatty acids in total; the content varies between samples of different species but is quite similar amongst samples of the same species. Fatty acids characterized by symbiotic microalgae 16:2n-7, 18:3n-6, 18:4n-3 Fatty acids 18:3n-6 were absent in *S. flexibilis* samples, minor content in *M. platyphylla* samples, and relatively significant in *Acropora* sp. (from 7.07% to 9.59% total fatty acids). Two fatty acids 16:2n-7 and 18:4n-3 were present in all 3 species, the highest in the sample *S. flexibilis*. The tetracosapolyenoic fatty acids 24:5n-6 and 24:6n-3 are soft coral marker fatty acids, present in only 5 samples of *S. flexibilis* with 24:5n-6 content ranging from 2.56% to 5.58% total fatty acids, 24:6n-3 is much lower (0.24% to 0.76%). The two PUFAs with the highest concentration were 20:4n-6 and 22:6n-3. 20:4n-6 was present in the total fatty acids of S. flexibilis with a quite high content (9.76% to 17.12% of the total axb), in contrast, the proportion was very low in five *Millepora* samples and five Acropora *samples*. 22:6n-3 was significantly high in the five *Millepora* samples (12.63% to 25.29% total fatty acids) and minor in the samples of the other two species.

Keywords: Lipid, fatty acids, symbiotic, coral, hydrocoral.

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https://doi.org/10.15625/1859-3097/21302
ISSN 1859-3097; e-ISSN 2815-5904/

INTRODUCTION

The phylum *Cnidaria* includes about 30,000 species; the most studied cnidarian species are corals (including hard corals (Hexacorralia), soft corals (Octocorallia)), anemones of class Anthozoa, hydrocorals (Hydrozoa) and jellyfish (Scyphozoa). Corals are rich in lipids, especially soft corals, the lipid content of which can account for up to 23% of the dry weight of coral soft tissue [1]. Lipids are the main source of metabolic energy and are essential for forming cell membranes and tissues. They are important in marine animals' physiology and reproduction and reflect the marine environment's distinctive biochemical and ecological conditions [2–4]. Total lipid content varies significantly depending on the coral's reproductive cycle, season of the year, habitat depth, light, water temperature, and other environmental factors [5]. Fatty acids are an essential component of the total lipids of organisms; they are present in almost all lipid classes. The interest of chemists, biochemists, and biotechnologists in marine lipids and fatty acids (FAs) has increased in recent years with the recognition that polyunsaturated fatty acids (PUFA) are important for human health and nutrition; they are necessary for reproduction and growth. The proportion and relative composition of FA in marine organisms are specific to each group and species, and depend on environmental conditions. Many comprehensive studies on marine fatty acids have been performed, addressing their occurrence, role, and the methods used in fatty acid analysis [6–9]. As a holobiont (ecological unit), the coral forms complex interactions with various microorganisms forming a critical part of the entire superorganism [10]. The most well-known coral symbionts are intracellular microalgae (the flagellated algae of the family Symbiodiniaceae). Thus, members of the symbiont will contribute to the composition of the total lipids of these species. Then, the lipid and fatty acid composition of the research objects will include the profile of lipids, fatty acids of invertebrates, and lipids of symbiotic microalgae. In this study, we will analyze the fatty acid composition of 15 samples belonging to 3 coral species typical for coral groups, including soft coral *Sinularia flexibilis*, hard coral *Acropora* sp., and hydrocoral *Millepora platypylla*, assessing the diversity of fatty acid compositions present as well as examining the individual characteristics of each species.

MATERIALS AND METHODS

Materials

Samples of *Sinularia flexibilis* coral (S1–S5), *Acropora* sp. (A1–A5), and *Millepora platypylla* hydrocoral (M1–M5) were collected in the waters of Nha Trang, Khanh Hoa in April and June 2021, at a depth of 3–8 m. Each species was collected 5 samples at positions at least 3 m apart. The sample was identified by Assoc. Prof. Dr. Hoang Xuan Ben and his colleagues, the specimen is kept at the Institute of Oceanography - Nha Trang.

Methods

Total lipid extraction method. The total lipids were extracted from the collected hydrocoral samples according to the method of Folch J. F., using the solvent system $CHCl_3$:MeOH ratio 2:1 by volume [11]. Fatty acids were methylated to the methyl ester form by the agent H_2SO_4/MeOH 2%. The temperature for metabolism was 80°C for two hours. Then, the mixture was analyzed on a GC 2010 (Shimadzu, Japan) with a SUPELCOWAX 10 chromatographic column (30 m × 0.25 mm × 0.25 µm, Supelco, USA) at 210°C, injector temperature and detector 240°C; and GC-MS 2010 Ultra (Shimadzu, Japan) with MDN-5s chromatographic column (30 m × 0.25 mm × 0.25 µm, Supelco, USA), temperature increments every 3°C from 210°C to 270°C, holding for 40 minutes at 270°C, injector and detector temperature 270 ºC. Data were obtained using the GCMS Solution software (LabSolution, Shimadzu). The spectra were compared with the NIST library (Shimadzu) and the FA mass spectra archives [12, 13].

RESULTS AND DISCUSSION

A total of 39 fatty acids were identified in 15 samples belonging to the 3 coral species studied. Detailed results on the composition and content of fatty acids are presented in Table 1. For *Sinularia flexibilis* samples, 26 fatty acids were identified, and 27 fatty acids were identified in the *Acropora* sp. Sample. *Millepora platyphylla* has the most diverse fatty acid composition, with 35 identified fatty acids.

Figure 1 shows that, in the group of *Sinularia* soft coral samples, the difference between the ratio of SFA saturated fatty acid content and PUFA polyunsaturated fatty acid content is much smaller than that of the other two groups of corals. In sample S5, the content of saturated and polyunsaturated fatty acids was almost the same (45.22 and 47.96%). In the *Acropora* coral group, saturated fatty acids predominated over polyunsaturated fatty acids; in samples A2 and A3 the ratio of SFA/PUFA was up to 4.05 and 4.10. This difference is reduced in the *Millepora* samples. In all 3 groups of corals and hydrocorals, fatty acids with a double bond account for only a small amount.

Figure 1 also shows the difference in the proportions of n-3 and n-6 fatty acids. In the *Millepora* hydrocoral samples, the total content of n-3 fatty acids was much higher than that of n-6 fatty acids. In the M2 model, the n-3/n-6 ratio goes up to 4.45. In contrast, in *Sinularia* soft coral and *Acropora* hard coral, the ratio of n-3 is lower than n-6, and the difference between the two fatty acid families is also less than in the *Sinularia* sample.

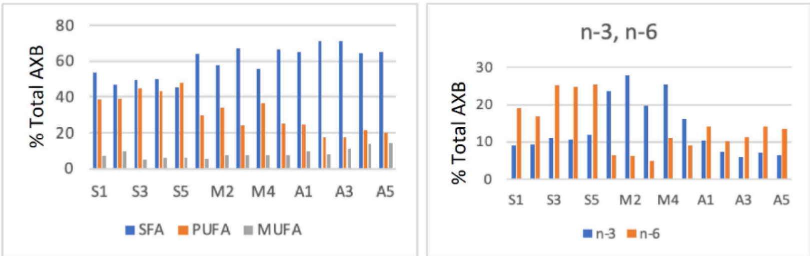

Figure 1. Contents of saturated fatty acid groups, PUFA bonds and 1 double bonds, n-3, n-6

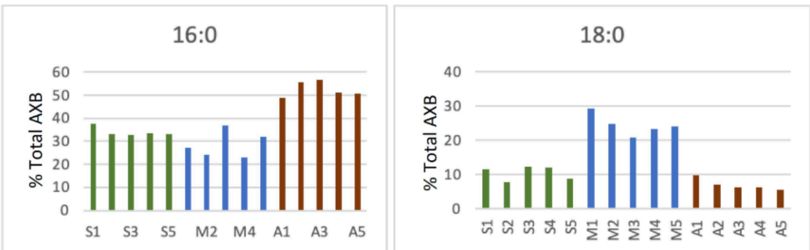

Figure 2. Fatty acid content 16:0, 18:0 in coral/hydrocoral samples

Among the saturated fatty acids, 16:0 and 18:0 are the two main fatty acids with the highest proportion of total fatty acids. The remaining saturated fatty acids were present in the samples in small amounts or were specific to each coral species. Fatty acids 14:0, 15:0, 20:0, and 22:0 were present in all samples, 20:0 was present in the *Millepora* sample higher than the other 2 species. 17:0, 19:0, and 21:0 were only present in *M. platyphylla* specimens. Building a graph of the content of two fatty acids, 16:0 and 18:0, shows the ratio of fatty acids in each coral/aquatic coral species, which has its characteristics and is relatively equal in each species (Fig. 2). The 16:0 content was very high in five *Acropora* samples (48.71 to 56.84% total fatty acids) and lower in the other two species.

All three species of corals/hydrocorals studied are species containing symbiotic microalgae. C16 and C18 double-linked fatty acids such as 16:2n-7, 18:3n-6, and 18:4n-3 are typical for symbiotic microalgae in corals. Fatty acid 18:3n-6 was not present in the *S. flexibilis* samples, was present in very small amounts in the *M. platyphylla* samples, and accounted for relatively significant concentrations in the *Acropora* sp. (ranging from 7.07% to 9.59% total fatty acids). Two fatty acids 16:2n-7 and 18:4n-3 were present in all 3 species, the highest in the sample *S. flexibilis*. Figure 3 shows the percentages of these fatty acids in the coral samples studied.

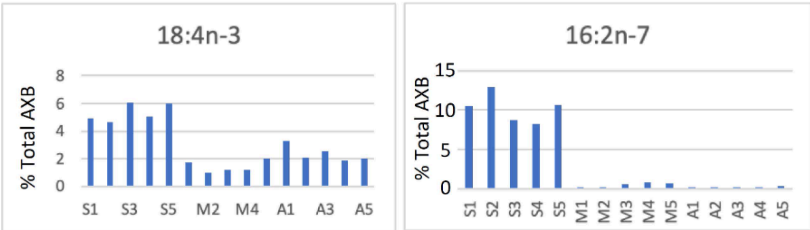

Figure 3. Fatty acid content of 16:2n-7, 18:4n-3 in coral/hydrocoral samples

The tetracosapolyenoic fatty acids 24:5n-6 and 24:6n-3 are marker fatty acids of soft corals. This group of fatty acids is not present in hard corals and aquatic corals, only in 5 samples of *S. flexibilis* with 24:5n-6 content ranging from 2.56% to 5.58% of total fatty acids, 24:6n-3 is much lower (0.24% to 0.76%). In a study on changes in the fatty acid composition of *S. flexibilis* species by months of the year by Imbs AB and DTPLy., the 24:5n-6 fatty acid content was also recorded at a higher level than the 24:5n-6 fatty acid content. 22:6n-3 in all months of the year [14].

The two PUFAs with the highest concentration were 20:4n-6 and 22:6n-3. 20:4n-6 was present in the total fatty acids of *S. flexibilis* with a quite high content (9.76% to 17.12% of the total axb); in contrast, the proportion was very low in 5 *Millepora* samples and 5 *Acropora* samples. 22:6n-3 was significantly high in the five *Millepora* samples (12.63% to 25.29% total fatty acids) and very low in the samples of the other two species (Figure 4). The significantly high 22:6n-3 ratio was also mentioned as a feature of the hydrocoral species *Millepora* in a study in 2021 by Imbs A. B. et al., [15], and the results were obtained. There are also similarities in this study.

Thus, the composition and fatty acid content of 15 samples belonging to 3 species containing symbiotic microalgae soft coral *Sinularia flexibilis*, hard coral *Acropora* sp., and the hydrocoral *Millepora platypylla* were analyzed. Most of the fatty acid groups were similar between samples of the same species. However, there were differences between samples of different species, further reinforcing that FA's relative proportion and composition in marine organisms are specific to each group or species. This study did not consider the effects of environmental conditions, reproductive cycles, or food source.

Table 1. Fatty acid composition of coral/hydrocoral samples

AXB	S1	S2	S3	S4	S5	M1	M2	M3	M4	M5	A1	A2	A3	A4	A5
12:0						0.1	0.07	0.14	0.12	0.3	0.28	0.1	0.41	0.18	0.29
14:0 iso						0.03	0.1	0.05	0.18	0.05					
14:2n-6											0.21	0.11	0.58	0.12	0.55
14:0 ante + 14:1n						0.02	0.08	0.08	0.11	0.02					
14:1n						0.06	0.02	0.08	0.04	0.08	0.51	0.13	1.06	0.24	1.06
14:0	1.82	3.99	1.39	1.63	1.57	2.91	3.1	3.91	2.2	4.98	4.9	4.52	6.66	5.81	7.33
15:0	1.17	0.31	1.43	1.07	0.89	0.21	0.48	0.38	0.53	0.35	0.09	0.59	0.12	0.15	0.21
16:2n-7	10.51	12.86	8.66	8.18	10.62	0.18	0.16	0.52	0.78	0.65	0.16	0.23	0.2	0.25	0.3
16:1n-9	3.87	5.67	2.45	3.05	2.98	0.21	0.91	0.68	1.17	0.19	2.57	1.93	3.24	4.03	4.56
16:1n-7						0.16	0.11	0.22	0.23	1					
16:0	37.76	33.01	32.67	33.38	33.09	27.02	24.19	37.01	22.97	32.2	48.71	55.6	56.84	51.05	50.73
7 Me 16:1n-10	0.76	0.96	0.56	0.56	0.53	0.42	0.56	0.56	0.45	0.41					
17:0						0.35	0.37	0.25	0.64	0.45					
18:3n-6						0.48	0.2	0.71	0.52	0.99	7.45	7.22	7.07	9.59	8.55
18:4n-3	4.88	4.64	6.04	5.05	5.98	1.73	1.01	1.18	1.22	2.02	3.27	2.08	2.58	1.89	2.03
18:2n-6	1.71	3.14	1.77	1.78	1.67	0.67	0.3	1.62	2.16	2.13	0.84	0.48	0.8	1.19	0.9
18:3n-3	0.57	0.35	0.63	0.57	0.67										
18:1n-9	3.00	3.69	2.31	2.53	2.76	4.23	5.1	5	4.07	4.31	5.07	5.22	5.42	7.4	7.22
18:1n-7	0.24	0.28	0.2	0.19	0.24	0.11	0.13	0.56	0.52	0.92	0.03	0.21	0.29	0.34	0.34
18:0	11.53	7.82	12.37	12.12	8.73	29.34	24.74	20.81	23.35	24.18	9.85	7.09	6.4	6.36	5.6
19:0						0.09	0.15	0.07	0.13	0.11					
20:4n-6	13.29	9.76	17.12	15.17	16.84	0.07	0.08	0.24	0.85	1.41	3.33	0.8	1.48	1.26	2.14
20:5n-3	1.25	0.89	1.6	1.37	1.42	0.69	0.55	0.47	0.77	0.81	3.6	1.72	1.65	1.76	2.23

AXB	S1	S2	S3	S4	S5	M1	M2	M3	M4	M5	A1	A2	A3	A4	A5
20:3n-6	0.89	0.58	0.99	1.25	0.76	0.04	0.03	0.09	0.1	0.08	1.01	0.05	0.73	1.49	0.9
20:4n-3	0.91	0.98	0.98	1.2	1.11	0.05	0.03	0.04	0.04	0.03	0.2	0.04	0.15	0.22	0.13
20:2n-6	0.24	0.5	0.23	0.33	0.22	0.02	0.01	0.06	0.07	0.07	0.13	0.03	0.06	0.1	0.05
20:1n-9	0.13	0.22	0.14	0.22	0.14	0.14	0.18	0.09	0.15	0.13	1.6	0.13	1.12	1.82	1.28
20:1n-7	0.06	0.04	0.02	0.02	0.09	0.02	0.01	0.01	0.02	0.02					
20:0	0.77	1.3	1.05	1.04	0.61	3.63	3.95	4.51	4.27	3.29	1.22	2.77	0.62	0.97	0.57
21:0						0.05	0.1	0.02	0.11	0.05					
22:5n-6	0.14	0.08	0.13	0.2	0.31	3.17	3.06	0.82	4.01	2.07		1.95			
22:6n-3	1.13	1.91	1.41	1.8	1.97	20.08	25.29	17.55	22.41	12.63	2.33	2.61	1.2	2.3	1.08
22:4n-6	0.14	0.18	0.33	0.35	0.68	1.85	2.42	0.72	2.62	1.73	1.11	1.5	0.45	0.36	0.46
22:5n-3		0.05	0.1	0.09		1.03	1	0.34	0.99	0.61	1.05	0.74	0.39	0.96	0.89
22:1n-9						0.44	0.76	0.11	0.98	0.38	0.09	0.52	0.03	0.03	0.07
22:0	0.41	0.51	0.41	0.52	0.33	0.35	0.51	0.07	0.83	0.34	0.16	0.53	0.05	0.07	0.06
24:5n-6	2.58	2.56	4.6	5.58	4.95										
24:6n-3	0.24	0.6	0.41	0.53	0.76										
24:0						0.05	0.09	0.01	0.17	0.03					
Total	100	96.88	100	99.78	99.92	100	99.85	98.98	99.78	99.02	99.77	98.9	99.6	99.94	99.53
Other	0	3.12	0	0.22	0.08	0	0.15	1.02	0.22	0.98	0.23	1.82	0.09	0.06	0.18
n-3	8.98	9.37	11.07	10.52	11.91	23.58	27.88	19.58	25.43	16.1	10.45	7.19	5.97	7.13	6.36
n-6	18.99	16.8	25.17	24.66	25.43	6.48	6.26	4.78	11.11	9.13	14.08	10.19	11.17	14.11	13.55
SFA	53.46	46.94	49.32	49.76	45.22	64.13	57.85	67.23	55.5	66.33	65.21	71.2	71.1	64.59	64.79
PUFA	38.48	39.08	45	43.45	47.96	30.06	34.14	24.36	36.54	25.23	24.69	17.61	17.34	21.49	20.21
MUFA	7.3	9.9	5.12	6.01	6.21	5.81	7.86	7.39	7.74	7.46	9.87	8.14	11.16	13.86	14.53

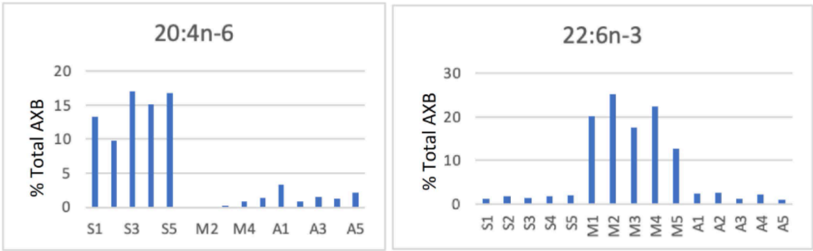

Figure 4. Fatty acid content of 20:4n-6, 22:6n-3 in coral/hydrocoral samples

CONCLUSION

In general, 15 coral samples belonging to 3 soft coral species *Sinularia flexibilis*, hard coral *Acropora* sp., and the hydrocoral *Millepora platypylla*, 39 fatty acids were identified in which *Sinularia flexibilis* sample identified 26 fatty acids, 27 fatty acids were identified in *Acropora* sp. samples, *Millepora platyphylla* has the most diverse fatty acid composition with 35 fatty acids. The analysis results show that among coral samples belonging to the same species, the composition and content of fatty acids are similar; on the contrary, there are certain differences between samples of different species, which reinforces the hypothesis that the proportion and relative composition of FA in marine organisms are specific to each group and species. This study has yet to address the effects of environmental conditions, reproductive cycles, or food sources, and we hope to have the conditions to conduct such studies in the future.

Acknowledgements: The research is funded by Project code number QTRU01.10/21–22 under the cooperation program with the Russian Basic Research Fund of Vietnam Academy of Science and Technology.

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