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Molecular species of some glycerophospholipid classes of soft coral *Sinularia leptoclados* collected in Nha Trang, Khanh Hoa

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ABSTRACT

In the soft coral *Sinularia leptoclados*, 30 molecular species belonged to 4 glycerophospholipid classes, including 8 ethanolamine glycerophospholipid (PE), 13 choline glycerophospholipid (PC), 3 serine glycerophospholipid (PS) and 6 inositol glycerophospholipid (PI) molecular species were identified. PE 18:1e/20:4, PC 18:0e/20:4, PS 18:0e/24:5 and PI 18:0/24:5 are the most abundant species with value of 69.94%, 45.57%, 68.55% and 68.18%, respectively. The PE, PC, and PS classes are reported to contain alkylacylphospholipid; meanwhile, a considerable level of diacylphospholipid is found in PI. A large portion of C20:4n polyunsaturated fatty acid was found in PE and PC; meanwhile, C16, C18, C22, and C24 fatty acids were reported at a minor level. The most dominant polyunsaturated fatty acid in PI and PS is tetracosapolyenoic C24. In the presence of fatty acids specific for the biosynthesis of zooxanthellae18:4n and 22:6n, PC is the most influenced class by the lipid composition of symbiotic microalgae. The PC 16:0e/18:4 and PC 18:1e/22:6 molecular species with recorded content of 1.69% and 8.05% are the evidence for lipid transportation from zooxanthellae to host corals. The PE, PS, and PI classes exhibit the lipid composition of the host coral; also, they are less affected by zooxanthellae lipids.

Keywords: Lipid, lipid molecular species, glycerophospholipid, *Sinularia leptoclados*.

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INTRODUCTION

Corals are the most studied species in the phylum Cnidaria, which includes about 30,000 species. In addition, there are sea anemones of the class Anthozoa, Hydrozoans (Hydrozoa), and jellyfish (Scyphozoa). In recent years, based on the advances in modern equipment and new methods, scientists have been able to quantify the molecular species of lipid classes qualitatively and accurately in corals, and the number of published works has increased significantly [1]. Most studies on coral are based on common species that make up the structural basis of coral reefs. The molecular species of lipids were analyzed in the stony coral *Acropora cereis* [2] and three soft species (*Sinularia* sp., *Sinularia heterospiculata* and *Sinularia siaesensis*) [3]. The composition of molecular species of wax esters was studied in reef-building corals (*Acropora acuminata*, *Acropora cytherea*, and *Turbinaria peltata*), soft tropical corals that do not contain symbiotic algae (*Mopsella spinosa*, *Menella flora*, and *Menella praelonga*) and soft tropical corals contain symbiotic microalgae (*Cladiella laciniosa*, *Sinularia brassica*, and *Sinularia robusta*) [4]. The triacylglycerol and monoalkyldiacylglycerol composition of the hydrocoral *Millepora platyphylla* has recently been published [5]. Several molecular species were identified in *Acropora cervicornis* [6] and *Pocillopora damicornis* [7]. Polar lipids in colonial stony coral *Seriatopo racaliendrum* [8, 9] cold water soft corals that do not contain the symbiotic microalgae *Gersemia rubiformis* [10], and some symbiotic tropical soft corals such as *Capnella* sp. [11], *Xenia* sp. [12], and *Sinularia macropodia* [11] were identified. In two symbiotic tropical hydrocorals, *Millepora dichotoma*, *M. Platyphylla*, and a symbiotic cold-water hydrocoral *Allopora steinegeri* [5, 13, 14], their molecular species were also reported. Most tropical corals contain intracellular symbiotic algae, most of which are unicellular algae of the genus Symbiodiniaceae (referred to as zooxanthellae). These microalgae are essential for the survival of the host corals, and the composition of the total lipids of these species will include both those of the host coral and symbiotic microalgae [15–17]. Despite the diverse data on fatty acids and lipid classes, the number of studies focused on molecular species of these invertebrates is still limited, especially in coral species. This study will report the molecular species of glycerophospholipid in soft coral *Sinularia leptoclados*.

MATERIALS AND METHODS

Materials

Samples of soft coral *Sinularia leptoclados* in Nha Trang bay, Khanh Hoa, colleced in 2020 at a depth of 7–8 m, were identified by Assoc. Prof. Do Cong Thung and colleagues at the Institute of Marine Environment and Resources, Hai Phong.

Research methods

Method to determine the molecular species of lipid classes: The molecular species of polar lipids in soft coral samples were analyzed using high-resolution mass spectrometry and recorded on a Shimadzu LCMS-IT-TOF instrument (Shimadzu, Kyoto, Japan). Analyzes were performed with a Shimadzu Prominence liquid chromatographic system. The phospholipid molecular species were identified by HRMS and determined by comparison with Shimadzu Solution standard spectrometers using analytical software v.3.60.361. The molecular species of each polar lipid class were quantified by the area of each peak [12].

RESULTS AND DISCUSSION

The primarily identified glycerophospholipids are ethanolamine glycerophospholipid, inositol glycerol-phospholipid, serine glycerophospholipid, and choline glycerophospholipid. These glycephospholipids contain choline, ethanolamine, serine, and inositol heads at the polar ends of their structures. Depending on the head at the sn-1 and sn-2 positions of the glycerol backbone, each glycerophospholipid can be 1,2-diacyl, 1-O-alkyl-2-acyl (plasmanyl) and 1-O-alkyl(-1-O-enyl)-2-acyl (plasmenyl, plasmalogen). In this study, the letter "e" denotes the head attached to the glycerol molecule with an ether bond. The fragmentation characteristics and molecular species identification methods of glycerophospholipid classes have been described in previous studies [2, 3, 12].

Molecular species of PE

In the ethanolamine glycerophospholipid (PE) class of soft coral *Sinularia leptoclados*, we identified 8 molecular species (Table 1), of which PE 18:1e/20:4are the most abundant (69.94%) (Fig. 1). There is the existence of fatty acids 20:4n, 24:5n, and 18:2n in PE. These are biomarkers of fatty acids that characterize the lipid composition of host corals in the coral-symbiotic relationship [18], in which 5/8 molecular species contain 20:4n fatty acids with a total content of 85.03%.

These results are similar to the obtained data in previous studies on the phospholipid molecular species of some Sinularia corals (*Sinularia macropodia* [11], *Sinularia siaesensis* [3], *Sinularia flexibilis* [19]) with high content of 20:4n fatty acids. PE 18:1e/20:4 is the major molecule in the PE class of *S. macropodia*, *S. siaesensis*, and *S. flexibilis*, containing of 56.0%, 42.54%, and > 50%, respectively. However, while diacyl PE is reported in all the mentioned coral species, alkylacylphospholipid is the only molecule determined in *S. leptoclados*. In addition to the high concentrations of 20:4n fatty acids, 24:5n fatty acids were present in minor amounts (5.17%), and 18:2n were present in trace amounts (0.18%). According to Imbs et al., C24 tetracosapolyenoic fatty acids are taxonomic features of soft corals; they commonly presented in the PE and PC classes with a content of less than 3%.

Table 1. Molecular species of PE in soft coral *S. leptoclados*

No.	Peak *m/z*	Molecular species	Molecular formula	DBE	Content (%)
1	806.5965	18:0e/24:5	$C_{47}H_{86}NO_7P$	7	0,98
2	804.5811	18:1e/24:5	$C_{47}H_{84}NO_7P$	8	4,19
3	750.5387	18:1e/20:4	$C_{43}H_{78}NO_7P$	7	69,94
4	752.5499	18:0e/20:4	$C_{43}H_{80}NO_7P$	6	3,80
5	722.5213	16:1e/20:4	$C_{41}H_{74}NO_7P$	7	0,33
6	778.5309	19:1/20:4	$C_{44}H_{78}NO_8P$	8	4,38
7	764.5543	19:1e/20:4	$C_{44}H_{80}NO_7P$	7	6,58
8	726.5491	18:1e/18:2	$C_{41}H_{78}NO_7P$	5	0,18
	Others				9,61

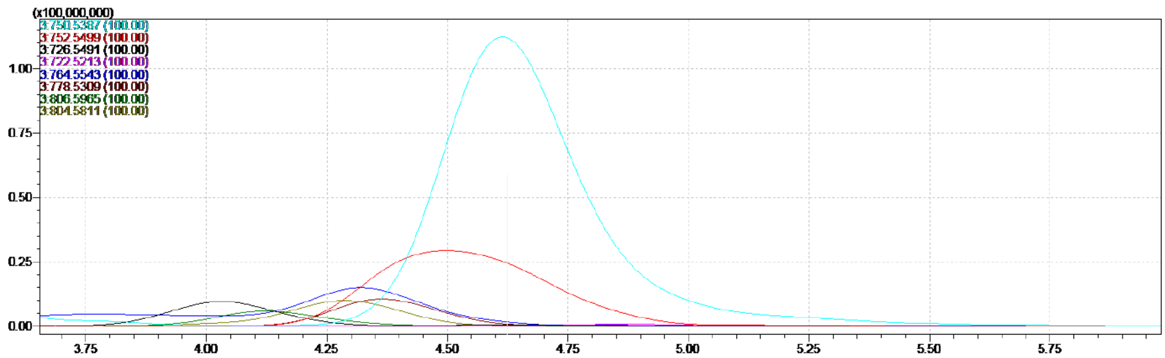

Figure 1. Negative-ions [M-H]⁻ HPLC/HR-ESI-MS chromatograms of PE in soft coral *S. leptoclados*

Molecular species of PC

Soft coral *S. leptoclados* contains the symbiotic algae zooxanthellae. Therefore, lipid extraction from *S. leptoclados* samples would consist of a lipid mixture of symbiotic microalgae and host coral. Zooxanthellae lipids can account up to 20% of total coral lipids [18], of which, 56–67% are polar lipids. In the polar lipid composition of zooxanthellae, in addition to glycolipids accounting for a significant portion (~55%), the choline glycerophospholipid class also accounts for a considerable amount (~19%), and this is one of the main lipid classes of symbiotic algae of corals. Thus, among the phospholipid classes, the composition of choline glycero-phospholipids of coral species containing symbiotic microalgae is mainly influenced by the lipids of zooxanthellae [12, 20].

Table 2. Molecular species of PC in soft coral *S. leptoclados*

No.	Peak *m/z*	Molecular species	Molecular formula	DBE	Content (%)
1	746.6086	18:1e/16:0	$C_{42}H_{84}NO_7P$	3	2,38
2	716.5581	16:0e/16:2	$C_{40}H_{78}NO_7P$	4	1,73
3	742.5748	16:0e/16:3	$C_{42}H_{80}NO_7P$	5	1,16
4	774.6369	18:0e/18:1	$C_{44}H_{88}NO_7P$	3	1,61
5	744.5997	16:0e/18:2	$C_{42}H_{82}NO_7P$	4	4,94
6	772.6220	18:0e/18:2	$C_{44}H_{86}NO_7P$	4	4,01
7	740.5887	16:0e/18:4	$C_{42}H_{78}NO_7P$	6	1,69
8	768.5883	16:0e/20:4	$C_{44}H_{82}NO_7P$	6	16,18
9	794.6022	18:1e/20:4	$C_{46}H_{84}NO_7P$	7	2,03
10	796.6156	18:0e/20:4	$C_{46}H_{86}NO_7P$	6	45,57
11	762.5621	16:1e/20:6	$C_{44}H_{76}NO_7P$	9	0,99
12	818.5922	18:1e/22:6	$C_{48}H_{84}NO_7P$	9	8,05
13	850.6376	18:0e/24:5	$C_{50}H_{92}NO_7P$	7	0,61
	Others				9,05

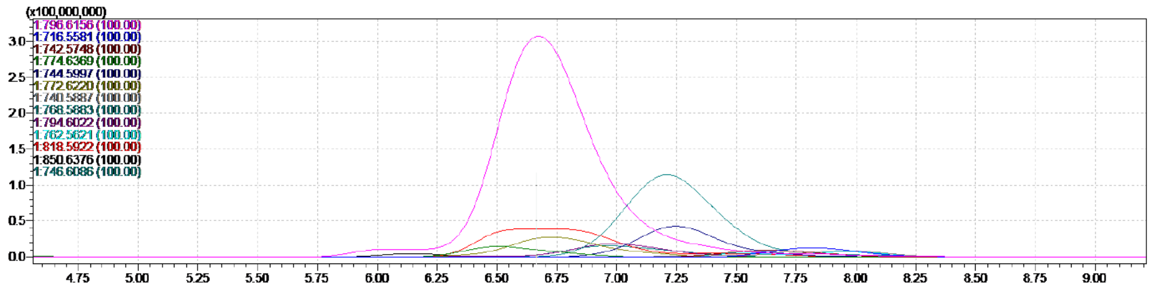

Figure 2. Positive-ions $[M+H]^+$ HPLC/HR-ESI-MS chromatograms of PC in soft coral *S. Leptoclados*

According to the obtained data, 13 molecules of PC have been identified in soft coral *S. leptoclados*, of which all of them are alkylacylphospholipid (Table 2). The dominant molecules in this class are PC 18:0e/20:4 (45.57%) and 16:0e/20:4 (16.18%) (Fig. 2). In two soft corals, *S. macropodia* and *S. siaesensis*, as reported previously, PC is also the most diverse in terms of the number of molecular species identified, in which PC 18:0e /20:4 and 16:0e/20:4 are the most abundant molecules in this class [3, 11]. Among the 13 identified molecules, the presence of fatty acids C16, C18, C20, C22, and C24 was more prevalent than that of the PE class. C20:4n remains predominant with the total content molecules containing this fatty acid accounting for 63.78%. These values in *S. macropodia* and *S. siaesensis* were 53.5% and 39.99%, respectively. C24:5n were determined only in PC 18:0e/24:5 with a minor content of 0.61%.In the composition of the PC, it should be noted the presence of PC 16:0e/18:4 and PC 18:1e/22:6. In previous studies, scientists have shown that 18:4n and 22:6nare the specific fatty acids for lipid biosynthesis of symbiotic algae of corals [18, 21]. However, in the lipids of zooxanthellae, only diacylphospholipids were identified [15, 16]. Themselves cannot synthesize alkylacylphospholipids which the biosynthesis takes place in host corals. Thus, although the content of PC 16:0e/18:4 and PC 18:1e/22:6 accounts for only 1.69% and 8.05%, this is a sign of the transfer of lipids from zooxanthellae to host corals, and host corals use these fatty acids to biosynthesize their lipids.

Molecular species of PS

Three molecules of PS were identified in soft coral *S.leptoclados*, in which the dominant molecules are PS 18:0e/24:5 (68.55%) (Table 3, Fig. 3). Similar to the PE and PC, only alkylacylphospholipid were recorded in the PS class of this species. In soft coral *S. Macropodia* and *S. siaesensis*, serine glycerophospholipid class with PS 18:0e/24:5 content up to 83.90% and 80.35% of the total classes [3, 11]. All the molecules of PS contain 24:5n and 24:6n tetracosapolyenoic fatty acids, which are specific for the biosynthesis of host coral lipids. Therefore, similar to the PE, serine glycerophospholipid is a typical class of host coral lipids and is less affected by the lipid composition of zooxanthellae.

Table 3. Molecular species of PS in soft coral *S. leptoclados*

No.	Peak *m/z*	Molecular species	Molecular formula	DBE	Content (%)
1	850.5913	18:0e/24:5	$C_{48}H_{86}NO_9P$	8	68,55
2	872.5723	20:3e/24:5	$C_{50}H_{84}NO_9P$	11	28,53
3	848.5759	18:0e/24:6	$C_{48}H_{84}NO_9P$	9	2,92

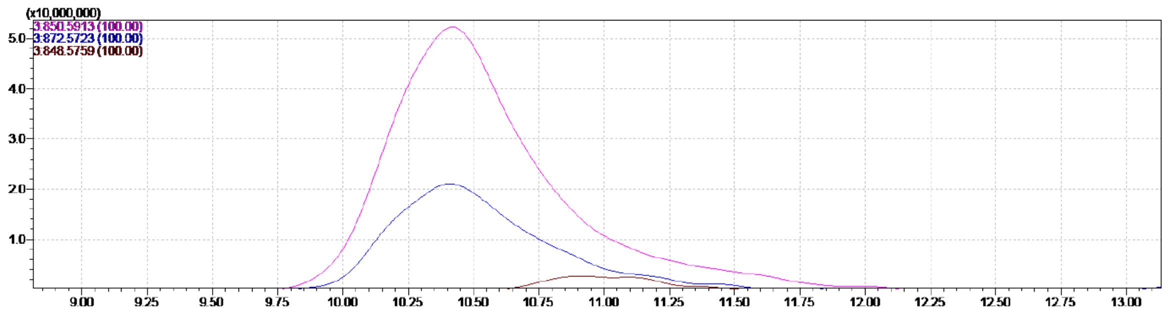

Figure 3. Negative-ions $[M\text{-}H]^-$ HPLC/HR-ESI-MS chromatograms of PS in soft coral *S. Leptoclados*

Molecular species of PI

It has been estimated that most of the characteristic variations are found in the molecular species of PI [11]. A total of 6 molecules of PI have been identified in soft coral *S.leptoclados* (Table 4, Fig. 4). In previous studies, it is reported that there were 4 and 6 molecules of PI found in *S. macropodia* and *S. siaesensis*, respectively. Although there is no significant difference in the number of identified molecules, there are specific differences in molecules of PI in the *Sinularia* species. In *S. macropodia* and *S. siaesensis* species, the dominant molecules are 3 diacyl phospholipids PI 18:0/22:4, PI 18:0/24:5, and PI 18:0/24:6. In *S.leptoclados* species, PI 18:0/24:5 accounted for 68.18%, the remaining two forms of alkylacyl phospholipid PI 18:0e/20:4 and 18:0e/24:5 also occupied significant content (8.10 and 14.16%). In two species, *S. macropodia* and *S. siaesensis*, most of the molecules are diacyl PI (100% and 98.9%); meanwhile, in

S. leptoclados, the total content of alkylacyl PI accounts for 22.25%. PI is the only glycerolipid class in this coral that contains diacylphospholipids. If the alkylacylphospholipids are fully biosynthesized molecules of the host coral, then the diacylphospholipids can be obtained from zooxanthellae lipids or food sources, etc. The long-chain fatty acid tetracosapolyenoic C24 is also widely distributed in this class, with the total content of molecules containing tetracosapolyenoic fatty acids up to 84.85%.

Table 4. Molecular species of PI in soft coral *S. leptoclados*

No.	Peak *m/z*	Molecular species	Molecular formula	DBE	Content (%)
1	911.5576	18:0/22:5	$C_{49}H_{85}O_{13}P$	9	1,40
2	913.5731	18:0/22:4	$C_{49}H_{87}O_{13}P$	8	3,08
3	937.5695	18:0/24:6	$C_{51}H_{87}O_{13}P$	10	2,51
4	939.5885	18:0/24:5	$C_{51}H_{89}O_{13}P$	9	68,18
5	871.5795	18:0e/20:4	$C_{47}H_{85}O_{12}P$	7	8,10
6	925.5552	18:0e/24:5	$C_{51}H_{91}O_{12}P$	8	14,16
	Others				2,57

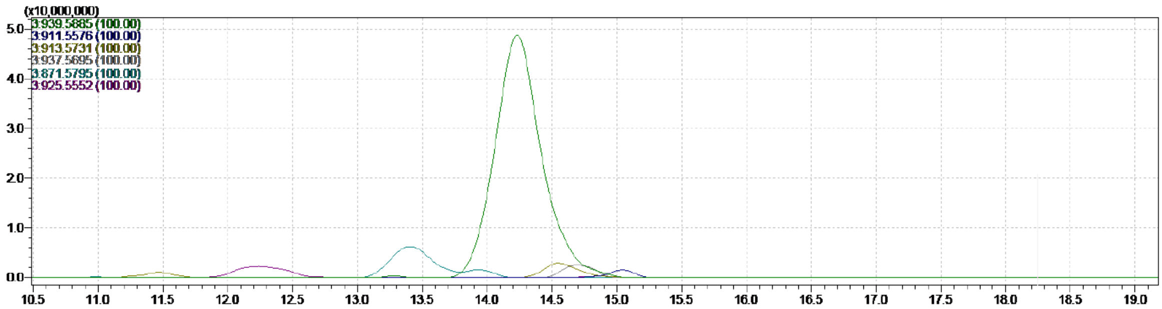

Figure 4. Negative-ions [M-H]⁻ HPLC/HR-ESI-MS chromatograms of PI in soft coral *S. leptoclados*

CONCLUSION

In a general analysis, 30 molecules belonging to 4 glycerophospholipid classes have been identified in soft coral samples, of which there are eight molecules of ethanolamine glycerophospholipid, 13 molecules of choline glycerophospholipid, three molecules of serine glycerophospholipid and six molecules of inositol glycerophospholipid. The PE, PC, and PS contain alkylacylphospholipids, while the diacylphospholipids are dominant in the PI class. C24 tetracosapolyenoic fatty acids, specific for lipid biosynthesis in host corals, are mainly concentrated in PS and PI. In PE and PC, C20:4n fatty acids are distributed with high content; there are also C16, C18, C22, and C24 with minor content. PC is reported to be influenced by the lipid composition of the symbiotic microalgae since molecules containing characteristic fatty acids are specific for the biosynthesis of zooxanthellae. PE, PS, and PI classes reflect the lipid composition of the host coral and are less affected by zooxanthellae lipids. PC 16:0e/18:4 and PC 18:1e/22:6 account for only 1.69 and 8.05%; however, this is evidence of lipid transportation from zooxanthellae to host corals.

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