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Comparison of the antibacterial activity, volatiles and fatty acid composition of lipids of *Phycopsis* species collected at different locations from the Bay of Bengal (Orissa coast)

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Abstract: The fatty acid composition as well as the volatiles and an antibacterial screening of the total lipids isolated from marine sponge *Phycopsis* sp. collected at two different locations from the Bay of Bengal of the Orissa coast having different morphological features were studied. The content of linear saturated acids was 30.25 % in *Phycopsis* sp. 1, while their content reached 50.33 % in *Phycopsis* sp. 2. The amount of monobranched, saturated acids was 44.87 % in *Phycopsis* sp.1 and 38.83 % in *Phycopsis* sp. 2. There was more phytanic acid (7.92 %) in *Phycopsis* sp. 2 than in *Phycopsis* sp. 1 (4.06 %). The amount of 5,9-pentacosadienoic acid was found to be 5.54 % in *Phycopsis* sp. 1, while it was absent in *Phycopsis* sp. 2. Both species showed differences in their fatty acid composition and volatiles as well as in the antibacterial screening of their lipid extracts.

Keywords: sponges; *Phycopsis* sp.; fatty acids; volatiles; antibacterial.

INTRODUCTION

Over the past decade, marine sponges from the class Demospongiae have attracted growing interest because of their unique chemical composition and biological activity. A number of biologically active secondary metabolites have been found in sponges.[1] Some unusual fatty acids (FAs) of sponges also exhibit biological activity.[2] Many new FAs were identified in sponge lipids, such as unsaturated FAs with an unusual distribution of the double bonds,[3] branched chain FAs[4] and FAs with unusual substituents in the carbon chain, such as the cyclopropane group,[5] methoxy group,[6] acetoxy group,[7] *etc*.

The bioactivity of the sponge *Phycopsis* sp. was already studied by Venkateswarlu *et al.* in 1995.[8] A CH_2Cl_2–MeOH extract of this organism exhibited antibacterial activity against *E. coli* and *B. subtilis* and a prenylated aromatic com-

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pound, phycopsisenone, was isolated from this extract. This paper presents the fatty acid composition, volatiles and antibacterial screening of lipid extracts against different pathogens of the two *Phycopsis* species having different morphological features, which were collected from the same depth but at different locations from the Bay of Bengal.

EXPERIMENTAL

Sponge material

Sponge specimens *Phycopsis* sp. 1 and 2 (Class: Demospongiae Sollas, order: Halichondrida Vosmaer, Family: Axinellidae Ridley and Dendy) were collected from 25 m depth from the Bay of Bengal of the Orissa coast during February–March, 2006 from a newly found ridge lineation (sp. 1 – 18° 57.294' N, 84° 44.057'E; sp. 2 – 18° 57.717'N, 84° 44.410'E).[9,10] The two samples were identified to genus level by Dr. P.A. Thomas, Ex-Emeritus Scientist (ICAR), Trivandrum, Kerala.

Extraction

Sponges were thoroughly washed and air-dried. Ten grams of each species was homogenised and successively extracted three times with chloroform–methanol (2:1, v/v) to isolate the lipids.[11] The crude lipid extracts were purified by a "folch wash"[12] to remove non-lipid contaminants. The chloroform phase was separated from the combined extract, dried over anhydrous sodium sulphate and concentrated under a nitrogen atmosphere. The yield of lipids was 4.0 % of the dry weight of the sponges.

Preparation of fatty acid methyl esters

The fatty acids so obtained were converted to the corresponding methyl esters. Fatty acids (10 mg) were dissolved in 4 ml of 5 % hydrochloric acid in methanol and 0.5 ml benzene and then the mixture was refluxed in a silicone bath at 80–100 °C for 2 h. After cooling, the methyl esters were extracted with petroleum ether, simultaneously neutralized and dried over a sodium sulphate–sodium bicarbonate mixture. The solvent was evaporated to dryness under reduced pressure at 40 °C in a water bath. These fatty acid methyl esters (FAME) were then analysed by GC/MS for identification.

Isolation and analysis of the volatile compounds

Part of the crude lipophilic extract (100 mg) was subjected to a 4-h distillation–extraction in a Lickens–Nickerson apparatus.[13] The volatiles were extracted from the distillate with diethyl ether (yield: 3 mg) and investigated using a Shimadzu QP-5000 GC-MS with a 25 m× ×0.25 mm, 0.25 μm film thickness, WCOT column coated with 5 % diphenyl siloxane, supplied by J & W (DB-5). Helium was used as the carrier gas at a flow rate of 1.2 ml/min, at a column pressure of 42 kPa. The column temperature was programmed from 40 to 280 °C at a rate of 4 °C/min. The ionization voltage (EI) was 70 eV.

Antibiotic activity testing of lipid extracts of the Phycopsis *species*

The antibacterial assay of the crude lipid extracts of *Phycopsis* sp. 1 and 2 (200 μg/6 mm disc) were performed against five fish pathogens (*Edwardsiella tarda*, *Staphylococcus aureus*, *Micrococcus* sp., *Pseudomonas aeruginosa* and *Escherichia coli*) and two human pathogens (*S. aureus* and *Salmonella typhi*), including three MDR (multi drug resistant) strains (*Staphylococcus pyogenes*, *Acinetobacter* sp. and *S. typhi*), by the disc-assay method.[14]

The test bacterial fish pathogen cultures were obtained from the stock cultures maintained in the Pathology Laboratory of Central Institute of Fresh Water Aquaculture, ICAR, Bhubaneswar.

Fatty acid methyl esters, FAME, analysis

FAME analyses were performed on a Shimadzu QP-5000 GCMS equipped with a mass selective detector and a 25 m×0.25 mm, 0.25 μm film thickness, WCOT column coated with 5 % diphenyl siloxane, supplied by J & W (DB-5). Helium was used as the carrier gas at a flow rate of 1.2 ml/min, at a column pressure of 42 KPa. The column temperature was programmed for fatty acid methyl esters (FAMEs) from 120–300 °C at a heating rate of 2 °C/min and 300 °C for 10 min, with a total run time of 100 min. An ionization voltage (EI) of 70 eV was used. Peak identification was performed by comparison of their mass spectra with those available in the Nist and Wiley libraries.

RESULTS AND DISCUSSION

Both *Phycopsis* species 1 and 2, collected at two different locations from a depth of 25 m from the Bay of Bengal of the Orissa coast, had different morphological features. *Phycopsis* sp. 1 was light orange in colour and had a finger-like structure (Fig. 1), while *Phycopsis* sp. 2 was light yellowish orange in colour with a rough body and soft structure (Fig. 2). The aim was to determine the difference in the fatty acid composition of the total lipids of two species of the same genus but having a different structural pattern. *Phycopsis* sp. 1 contained a greater number of fatty acids in comparison to *Phycopsis* sp. 2 (see Table I).

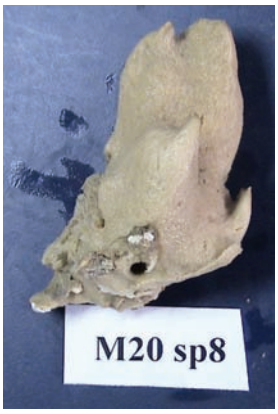

Fig. 1. *Phycopsis* sp. 1.

Fig. 2. *Phycopsis* sp. 2.

The whole series of linear saturated fatty acids from C14 up to C32 were revealed in the lipid composition of the sponges. The content of linear saturated fatty acid was 30.25 % in *Phycopsis* sp. 1, while the content was 50.33 % in *Phycopsis* sp. 2. All acids from C14:0 to C23:0 were found in *Phycopsis* sp. 1, except C19 and C22:0, among which C16:0 and C18:0 were dominant (11.76 and 9.29 %, respectively), while in *Phycopsis* sp. 2, except for C17:0, C19:0 and C22:0, all acids were present from C14:0–C23:0, among which C16:0, C18:0 dominated (8.95 and 8.88 %, respectively). The content of C21:0 was only 3.89 % in *Phycopsis* sp. 1, while in *Phycopsis* sp. 2, it was present in a reasonable amount (7.63

%). The total amount of tricosanoic acid was only 0.53 % in *Phycopsis* sp. 1, while its amount in *Phycopsis* sp. 2 was 6.02 %.

TABLE I. GC/MS analysis of the FAME of the total lipid of *Phycopsis* sp. collected at two different locations

Acid	Retention time min	Acid content, %	
		Phycopsis sp. 1	*Phycopsis* sp. 2
Tridecanoic acid, 3-methyl (C13:0, br[a])	4.21	2.24	–
Nonanoic acid (C9:0)	5.52	–	4.18
Tetradecanoic acid (C14:0)	7.75	2.35	2.64
Pentadecanoic acid (C15:0)	8.73	7.64	9.39
Tetradecanoic acid, 12-methyl (C14:0, br)	8.85	2.81	–
9-Hexadecenoic acid (C16:1)	10.55	1.14	–
Hexadecanoic acid (C16:0)	10.89	11.76	8.95
2-Hexylcyclopropaneoctanoic acid	11.49	5.34	6.68
14-Methylhexadecanoic acid (C16:0, br)	11.56	7.92	–
Heptadecanoic acid (C17:0)	11.87	2.58	–
3,7,11,15-Tetramethylhexadecanoic acid	13.45	4.06	7.92
15-Octadecenoic acid (C18:1)	13.62	–	2.86
10-Octadecenoic acid (C18:1)	13.63	3.77	–
Octadecanoic acid (C18:0)	13.94	9.92	8.88
11-Methyloctadecanoic acid	14.52	–	24.96
17-Methyloctadecanoic acid	14.55	25.03	–
2-Octylcyclopropaneoctanoic acid	15.20	1.53	–
Eicosanoic acid (C20:0)	16.79	1.90	2.64
16-Methylheptadecanoic acid	17.65	–	7.19
Heneicosanoic acid (C21:0)	17.79	3.89	7.63
Tricosanoic acid (C23:0)	20.26	0.53	6.02
5,9-Pentacosadienoic acid (C25:2)	22.21	5.54	–

[a]Branched

The total content of monobranched, saturated acids was 44.87 % of total FA content in *Phycopsis* sp. 1, while its amount was less in *Phycopsis* sp. 2 (38.83 %).

17-Methyloctadecanoic acid represented 25.03 % of the total monobranched FAs in *Phycopsis* sp. 1, while in *Phycopsis* sp. 2 11-methyloctadecanoic acid was dominant in the total monobranched FAs (24.96 %). 14-Methylhexadecanoic acid was also present in *Phycopsis* sp. 1 in a significant amount (7.92 %), while in Phycopsis sp. 2, 16-methylheptadecanoic acid was present in a significant amount (7.19 %). The amount of 2-hexylcyclopropaneoctanoic acid was found to be 5.34 % in *Phycopsis* sp. 1, while in *Phycopsis* sp. 2 it was 6.68 %. 2-Octylcyclopropaneoctanoic acid, was found in *Phycopsis* sp. 1, while it was absent in *Phycopsis* sp. 2.

The polymethyl branched saturated FAs of the sponges were represented by the usual isoprenoid FAs, 4,8,12-trimethyltridecanoic acid, phytanic acid and pristanic acids, their contents varying from 0.5 up to 20 % of the total FAs. Only

one isoprenoid fatty acid, *i.e.*, phytanic acid, was found in both species (4.06 and 7.92 % in species 1 and 2, respectively).

Monoenes are reported to vary from 2 to 50 % of the FA total content in various sponge species.[15] However, only two monoenes were found in *Phycopsis* sp. 1 and only one in *Phycopsis* sp. 2. Among the six isomers of the C16:1 acid, C16:1Δ9 prevailed in most of the species and its relative content was about 3–5 % on average.[15] In *Phycopsis* sp. 1, the C16:1Δ9 content was 1.14 % but it was not found in *Phycopsis* sp. 2. The content of C18:1Δ10 was found to be 3.77 % in *Phycopsis* sp. 1, but it was also absent in *Phycopsis* sp. 2. In *Phycopsis* sp. 2 only one monoenic acid was found and that was C18:1Δ15 (2.86 % of the total FA content), but it was absent in *Phycopsis* sp. 1.

In some species of sponges, polyenic FAs are represented exclusively by dienes. Thus, the ratio of dienes can reach 6–12 % of the FA total, *e.g.*, in the sponges *Agelas dispar*, *Anthosigmella varians* and *Chondrilla nucula*.[16] The amount of 5,9-pentacosadienoic acid was found to be 5.54 % in *Phycopsis* sp. 1 but it was absent in *Phycopsis* sp. 2.

Volatile compounds

Volatile compounds often possess valuable biological activities. They serve as allelochemicals defending the organism from bacteria, fungi and viruses. Analogous to other investigated sponges,[17,18] the volatiles in both *Phycopsis* sp. 1 and 2 appeared to be relatively simple (see Table II). *Phycopsis* sp. 2 contained no saturated *n*-hydrocarbons, while the amount of saturated *n*-hydrocarbons was 16.55 % in *Phycopsis* sp. 1. A number of saturated aliphatic aldehydes were found in *Phycopsis* sp. 2, whereas no such aldehydes were found in *Phycopsis* sp. 1. Significant concentrations of these compounds are an indication for the participation of bacteria (coming from the diet or associated with the tissues) in their formation.[18] 1,2-Benzenedicarboxylic acid, dioctyl ester constituted 89.49 % of the total volatiles content of *Phycopsis* sp. 2, which was absent in *Phycopsis* sp. 1. Only one ketone, *i.e.*, (*E*)-hept-3-en-2-one, was found in *Phycopsis* sp. 2, which was absent in *Phycopsis* sp. 1. Dodecan-1-ol was the only alcohol found in *Phycopsis* sp. 1, whereas *trans*-2-decenol, an unsaturated alcohol, was found in *Phycopsis* sp. 2. The volatiles in both the species collected at two different locations were found to differ significantly from each other.

Antibacterial activity

Antibacterial screening of the lipid extract of both species *Phycopsis* sp. 1 and *Phycopsis* sp. 2 were performed against different pathogens. The lipid extract of *Phycopsis* sp. 1 exhibited trace activity against only *Escherichia coli* (fish pathogen), while that of *Phycopsis* sp. 2 showed trace activity against four pathogens, *i.e.*, *Staphylococcus aureus* (human pathogen), *S. aureus* (fish pathogen), *Micrococcus* sp. and *E. coli* (Fish pathogen).

TABLE II. Composition of the volatile compounds in *Phycopsis* sp. collected at two different locations

Volatiles	Content, %	
	Phycopsis sp. 1	*Phycopsis* sp. 2
Pentanal	–	0.28
Hexanal	2.2	0.72
(*E*)-Hept-3-en-2-one	–	5.31
Heptanal	–	1.48
Undecane	11.24	–
Undecanal	1.98	–
Dodecane	5.31	–
Dodec-1-ene	16.08	1.25
trans-2-Decenol	1.24	–
Dodecan-1-ol	29.39	–
Non-2-enal	1.53	–
Nonanal	–	2.91
1,2-Dichlorohexane	7.43	–
1,2- Benzenedicarboxylic acid, dioctyl ester	–	89.49
Butane, 1-(2,2-dichloro-3-ethylcyclopropyl)	–	3.79
Dihydrocitronellol	18.62	–

CONCLUSIONS

From the above studies, it can be concluded that the two specimens of the *Phycopsis* genus, collected from same depth but at different locations and having different morphological features, differ in their volatiles and composition of fatty acids, as well as in the antibacterial activity of their lipid extracts.

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И З В О Д

ПОРЕЂЕЊЕ АНТИБАКТЕРИЈСКЕ АКТИВНОСТИ И САДРЖАЈА ИСПАРЉИВИХ МАТЕРИЈА И МАСНИХ КИСЕЛИНА У МАСТИМА ВРСТА *Phycopsis* САКУПЉЕНИХ НА РАЗЛИЧИТИМ ЛОКАЦИЈАМА БЕНГАЛСКОГ ЗАЛИВА (ОБАЛА ДРЖАВЕ ОРИСА, ИНДИЈА)

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Испитиван је садржај масних киселина и испарљивих материја, као и антибактеријска активност укупних масти изолованих из морских сунђера *Phycopsis* sp. различитих морфолошких карактеристика, сакупљених на две различите локације у Бенгалском заливу, обала државе Ориса, Индија. Садржај линеарних засићених киселина у *Phycopsis* sp. 1 износио је 30,25 %, док њихов садржај у *Phycopsis* sp. 2 достиже 50,33 %. Садржај моноразгранатих засићених киселина у *Phycopsis* sp. 1 и sp. 2 износио је 44,87%, односно 38,83 %. Садржај

фитанске киселине био је већи у *Phycopsis* sp. 2 (7,92 %) него у *Phycopsis* sp. 1 (4,06 %). Количина 5,9-пентакозадиенске киселине износила је 5,54 % у *Phycopsis* sp. 1, док у *Phycopsis* sp. 2 ова киселина није нађена. Ове врсте разликују је по садржају масних киселина и испарљивих материја, као и по антибактеријској активности липидних екстраката.

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