Malays. Appl. Biol. (2016) 45(2): 23–28

FATTY ACIDS AS BIOLOGICAL MARKERS FOR SYMBIOTIC BACTERIA IN *Phyllidia varicosa* AND *Phyllidiella pustulosa*

ALI ALQUDAH[1*], SHAHBUDIN BIN SAAD[1], DENY SUSANTI[1] and NOOR FAIZUL HADRY[2]

[1]*Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan Pahang, Malaysia*
[2]*Kulliyyah of Engineering, International Islamic University Malaysia, Kuala Lumpur, Malaysia*
[*]*E-mail: ali_quda@yahoo.com*

Accepted 13 October 2016, Published online 21 December 2016

ABSTRACT

The fatty acid (FA) composition of *Phyllidia varicosa* and *Phyllidiella pustulosa* (notum and viscera) was investigated. Samples were collected from coastal water of Balok - Pahang - Malaysia. This study was conducted to test the hypothesis that nudibranchs species host symbiotic bacteria by using fatty acids as biological markers. A high level of fatty acids group specific to the bacteria were detected in the selected species that called odd- branched chain fatty acids. Among them, high levels of iso- anteiso-C15:0, C15:0, iso-C16:0, C17:0, iso-C17:0, iso C17:1(n-5), iso C18:0, 14-methyl-C18:0 and iso-C18:0) were found and their percentages in the notum are significantly different compared to viscera. The total odd- branched chain fatty acids were 29.64% in *P. varicosa* and 30.66% in *P. pustulosa* compared to another group of fatty acids such as saturated FA, monounsaturated FA and polyunsaturated FA. The present study deals with the identification of cyclopropane FA in the nudibranch tissue for the first time which cyclopropaneoctanoic acid 2-hexyl and cyclopropaneoctanoic acid 2-octyl were detected. We suggest that symbiotic bacteria associated with the nudibranchs tissue originate these fatty acids.

Key words: *Phyllidia varicosa*, *Phyllidiella pustulosa*, fatty acids, biological markers, symbiotic bacteria

INTRODUCTION

The dorid nudibranchs *P. varicosa* and *P. pustulosa* are common tropical Indo-Pacific nudibranchs species (Brunckhorst, 1993). Both species occupy essentially similar feeding niches from protected to exposed rocky sites, where they are usually found from the low intertidal to 10m depth. Nudibranchs species are apparently vulnerable to the predators due to the shell is completely absent, and no obvious morphological defense structure against predators. *P. varicosa* and *P. pustulosa*, like many other nudibranch mollusks, feed on sponges. *P. varicosa* has been reported to feed on Halichondria sponge *Axinyssa aculeate* (Yasman *et al.,* 2003) while *P. pustulosa* feed on *Phakellia carduus* (Wright, 2003).

The biology and ecology of nudibranchs species, in particular, their food preferences, are inadequately investigated. Investigation of the fatty acid composition of marine invertebrates could be beneficial in elucidating their habits and food preferences. Fatty acids are vulnerable to change its composition depending on food availability, nutrient habits and physiological conditions of an organism. For instance, the lipid composition of the *Phyllidia coelestis* and *Chromodoris* sp. includes a significant amount of very long-chain fatty acids specific to sponges called demospongic acids in reference to their feeding ecology (Zhukova, 2007, 2014). Zhukova & Eliseikina (2012) have detected the existing of odd-numbered carbon chain and branched fatty acids, iso- and anteiso- that are specific for bacteria in *Dendrodoris nigra* tissue. The source of the bacterial fatty acids in marine organisms' tissue is presumed to be of microbial origin, which can be from their diet or acquired from symbiotic microorganisms. Marine invertebrates like sponges and ascidians host cyanobacteria, nonphototrophic bacteria, and algae associated with the intercellular matrix (Dalsgaard *et al.,* 2003).

Fatty acids have been used as a biomarker to reveal the symbiotic relationship between bacteria and bivalve mollusks (Zhukova *et al.,* 1992),

* To whom correspondence should be addressed.

tubeworms (Pond *et al.,* 2002), gastropods (Pranal *et al.,* 1996; Saito & Hashimoto, 2010) and amphipods (Fallick & Sargent, 1997). Zhukova & Eliseikina (2012) have reported that marine nudibranchs harbor symbiotic bacteria and was tested using analyses of fatty acids as biochemical markers and transmission electron microscopy of the tissues of *Dendrodoris nigra*. There are two alternative ways for nudibranchs to acquire odd-branched chain Fatty acids. First, nudibranchs are specialist feeders on sponge which are rich in bacteria. It is reasonable to presume that they may originate from the bacterial association with sponge. Second, based on the aberrant level of the bacterial fatty acids, we hypothesized that there is a symbiotic bacterial association with nudibranchs. The odd-branched chain fatty acids are predominant in bacteria (Gillan & Johns, 1986; Perry *et al.,* 1979), and have already been utilized as bacterial markers in marine food webs (Kamenev, 1995; Klussmann-Kolb & Brodie, 1999). The objectives of this study were to examine the fatty acids composition as biological markers and to determine the origin of bacterial fatty acids in the studied nudibranch species.

MATERIALS AND METHODS

Site and samples

Specimens of *P. varicosa* and *P. pustulosa* were collected from coastal water of Balok – Pahang "3°56.233' N 103°22.627° E" – Malaysia (Fig. 1). The collected nudibranchs were immediately placed in a plastic box with seawater from the site and equipped with live rock as a substrate and aeration pumps and then transported to the laboratory. The collected nudibranchs species were identified through the colouration pattern of their mantle and external morphology. Three batches of each species were used for lipid analysis.

Fatty acid analysis

The nudibranchs were dissected and was viscera separated from the notum. Fatty acids were extracted from freeze dried nudibranchs samples according to the method from Püttman *et al* (1993) for the qualitative and quantitative examination. Total lipid extract was placed in a screw-capped glass test tube and dissolved in 0.20 ml of toluene. The extracted fatty acids were transesterified into fatty acid methyl esters (FAME) using 1.5 ml of the methanol and 0.3 ml of HCl were added to the fatty acid extract acid at 95ºC for about an hour using water bath. After this treatment, purified water and hexane were added and the upper organic layer was transferred to a vial (Ichihara & Fukubayashi, 2010). This step was repeated several times to achieve complete extraction of FAME for viscera samples only. Samples were then dried and dissolved again in 20 µl hexane to get 50 times concentration and to remove all solvent peaks (toluene). The concentrated fatty acids (FAMEs) were then injected into gas chromatography–mass spectrometry (GC-MS) for analysis. Percentage of fatty acids detected in each treatment was expressed in Mean±SD.

Gas chromatography – mass spectrometry conditions

FAMEs were separated and quantified by Agilent 6890 N gas chromatography coupled with Agilent MS-5973 mass spectrometry selective detector (Agilent Technologies, USA, serial number. US14113031). FAME samples were injected by

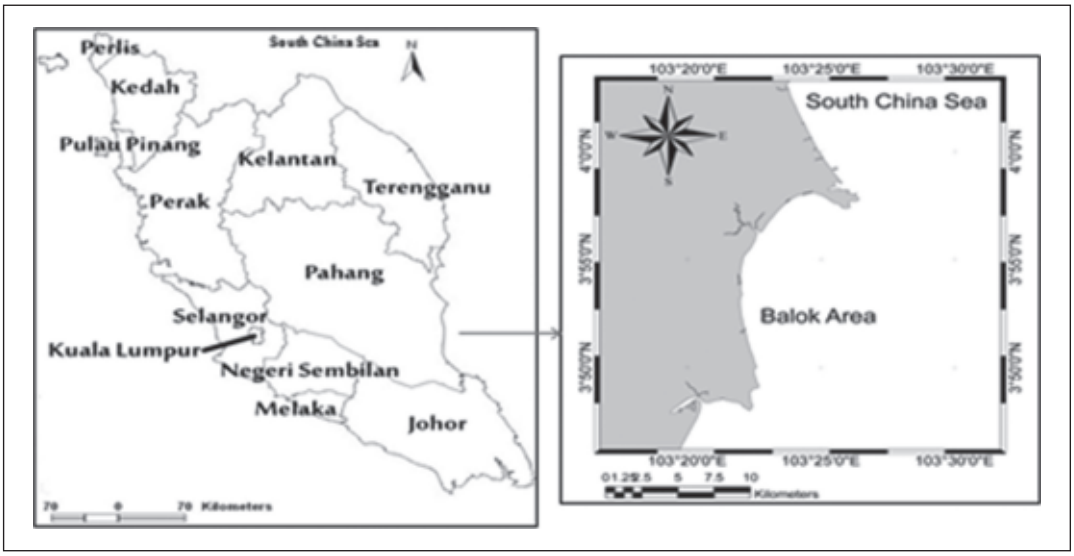

Fig. 1. Map of the Coastal water of Balok, Pahang, Malaysia.

using an Agilent autosamplier 7683 series injector onto the HP-5MS column (Agilent 19091S-433, 30 m dimension, 0.25 mm i.d., 0.25 m film thickness). Initially, the oven temperature was maintained 150ºC for 4 min and programmed 4ºC /min up to 325ºC, then kept at 250ºC for 5 min. The injector port and detector temperatures were held at 240 and 260ºC, respectively. Helium gas was used as a carrier at a flow rate of 1.0 ml/min and the split ratio was 1:50. A 70 eV electron impact (EI) in 50–550m/z scan range was applied to the running of the mass spectrometer (Kandhro *et al.,* 2010). The fatty acid components were recognized by comparing the MS spectrum of the component of fatty acid with standard library (Wiley Registry of Mass Spectral data).

Statistical analyses

The statistical analyses of percentages of fatty acids were tested by an analysis of fatty acid components among the nudibranch species (notum and viscera) using a Student's t test. Differences in means were considered statistically significant at $p < 0.05$.

RESULTS

The most predominant fatty acids in *P. varicosa* and *P. pustulosa*, occurring in concentrations exceeding 5% of the total, were C16:0 (7.67%), C15:0 (7.03%), C17:0 (6.85%), C18:0 (7.01%), C18:1n-9 (6.1%), C20:1 n–9 (5.52%) C20:4 n-6 (8.02%), representing 48.2% of the total fatty acids in notum of *P. varicosa*. In *P. pustulosa*, the prevalent percentages of the most common fatty acids were found as follow C16:0 (5.93%), C15:0 (6.51%), C17:0 (5.72%), C18:0 (6.19%), C18:1n-9 (5.87%), C20:1 n–9 (5.2%), and C20:4 n-6 (5.92%), accounting for 41.34% of the total fatty acids composition. The percentages of omega 3 polyunsaturated fatty acids (PUFA) that included C16:3(n-3), 20: 5(n-3), and 22:6(n-3) in total of 6.68% in *P. varicosa* and 6.4 % in *P. pustulosa* was lower than that of omega-6 PUFA (11.00% and 10.48%) in *P. varicosa* and in *P. pustulosa* respectively. Meanwhile, the level of omega-3 was slightly higher in notum compared to viscera in *P. pustulosa* (Table 1).

The selected nudibranch species exhibited an aberrant level of the odd-chain and branched fatty acids (OBFA). Among them, high level of odd-numbered fatty acids (e.g., anteiso-C15:0, C15:0, iso-C16:0, C17:0, iso-C17:0, iso C17:1(n-5), iso C18:0, 14-methyl-C18:0 and iso-C18:0). Another unique feature of the fatty acids composition of the *P. varicosa* and *P. pustulosa* were the presence of 2-octyl-cyclopropaneoctanoic and 2-hexyl-cyclopropaneoctanoic acids in the notum with trace concentrations. In total, these OBFA in *P. varicosa* and *P. pustulosa* represented 29.64% and 30.66% respectively, of the total fatty acids.

Monounsaturated fatty acids (ΣMUFA) were the second dominant group in *P. varicosa* and *P. pustulosa* with 25.46% to 24.11% respectively. Of all MUFA, oleic acid (C18:1 (n-9)) was the dominant MUFA, followed by C20:1 (n–9). The proportion of saturated fatty acids (ΣSFA) was low in the sea slug compared to the other fatty acids proportions. PUFA were presented mainly by C20:4 (n-6), C20:5 (n-3) and the dienoic acids C25:2 Δ5, 9. Some variations in the fatty acid groups found between tissues of *P. varicosa* such as OBFA were obviously different between the notum and viscera with 29.64% and 24.79% respectively. The amounts of odd-numbered branched fatty acids (15:0 and 17:0, iso and anteiso) in the notum are higher than the amounts in the viscera of the studied species.

DISCUSSION

The fatty acid composition of *P. varicosa* and *P. pustulosa* showed diverse numbers of fatty acids classes: saturated, monounsaturated and poly-unsaturated, even and odd, branched (iso- and anteiso-), short-chain and very long-chain fatty acids. The objective of this study was not to undertake an extensive study on lipid analysis but rather to find whether there are symbiotic bacteria that exist in the host tissue or not. A fatty acid analysis has been increasingly employed in detecting bacterial markers that may elucidate contributions by symbiotic bacteria to the diet and feeding ecology of the host organism (Goffredi *et al.,* 2005; McKenzie *et al.,* 2000; Stowasser *et al.,* 2012). The fatty acid composition of two sea slugs has a unique feature, a higher percentage of the odd chain fatty acids (C15, C17, and C19) and branched (iso- and anteiso-) fatty acids, and these fatty acids are specific for bacteria. Bacteria are the main organisms generally reported to produce large concentrations of iso-, anteiso-, monomethyl branched fatty acids (Gillan *et al.,* 1988). In particular, the odd-chain length 15:0 and 17:0 acids with iso- and anteiso- forms are commonly considered distinctive contents of bacteria (Gillan & Johns, 1986; Kaneda, 1991) and these fatty acids can be found in the areas of elevated bacterial activity. Early studies suggested that specific fatty acids present in marine organisms originate only from bacteria (Gillan *et al.,* 1988). High level of these compounds have been also detected in *Dendrodoris nigra* (Zhukova & Eliseikina, 2012)

Table 1. The percentages of fatty acid composition of *P. varicosa* and *P. pustulosa*. Results are expressed as the mean ± SD of three replicates

Fatty acids	*P. varicosa*		*P. pustulosa*	
	Viscera (mean* ± S.D)	Notum	Viscera (mean* ± S.D)	Notum
SAFA				
C 12:0	0.9 ± 0.1	–	1.9 ± 0.01	2.25 ± 0.07
C 14:0	1.57 ± 0.5	1.27 ± 0.4	2.63 ± 0.15	3.48 ± 0.16
C 16:0	7.11 ± 1.33	7.67 ± 0.38	6.16 ± 1.93	5.93 ± 1.09
C 18:0	6.01 ± 1.81	7.01 ± 0.41	7.01 ± 0.81	6.19 ± 0.23
C 20:0	3.01 ± 0.83	3.46 ± 0.53	4.01 ± 0.83	3.27 ± 0.13
C 22:0	2.19 ± 0.94	1.09 ± 0.14	3.49 ± 0.24	1.02 ± 0.02
ΣSAFA	20.79 ± 4.25	20.5 ± 3.45	25.2 ± 1.22	22.41 ± 2.52
	MUFA	MUFA	MUFA	MUFA
C 16:1(n-7)	3.4 ± 1.1	3.75 ± 0.5	1.91 ± 0.1	1.45 ± 0.05
C 18:1(n-11)	1.1 8 ± 0.55	1.98 ± 0.05	2.41 ± 0.08	2.27 ± 0.13
C 18:1(n-10)	3.17 ± 0.95	2.7 ± 0.45	3.72 ± 0.95	3.44 ± 0.81
C 18:1(n-9)	6.56 ± 1.92	6.1 ± 1.01	5.13 ± 0.52	5.87 ± 0.71
C 18:1(n-8)	6.45 ± 0.98	2.59 ± 0.09	3.52 ± 0.48	3.43 ± 0.3
C 19:1(n-9)	2.03 ± 0.51	1.63 ± 0.11	1.63 ± 0.21	1.5 ± 0.09
C 20:1(n-9)	4.22 ± 0.17	5.52 ± 0.17	5.24 ± 0.47	5.2 ± 0.21
C 22:1(n-9)	1.53 ± 0.62	1.19 ± 0.15	0.83 ± 0.12	0.95 ± 0.1
ΣMUFA	28.54 ± 3.21	25.45 ± 3.65	24.39 ± 1.02	24.11 ± 4.19
	PUFA	PUFA	PUFA	PUFA
C 16:3(n-3)	1.09 ± 0.15	0.69 ± 0.08	1.01 ± 0.11	1.1 ± 0.09
C 18:2(n-7)	2.44 ± 0.32	2.57 ± 0.2	3.54 ± 0.42	3.37 ± 0.42
C 18:2(n-3)	2.18 ± 0.71	2.98 ± 0.1	3.21 ± 0.11	3.56 ± 0.12
C 20:4(n-3)	9.52 ± 1.61	8.02 ± 0.61	6.42 ± 0.11	5.92 ± 0.65
C 20:5(n-3)	4.57 ± 0.53	4.17 ± 0.33	3.27 ± 0.43	3.39 ± 0.11
C 22:6(n-3)	2.02 ± 0.35	1.82 ± 0.37	2.02 ± 0.34	1.91 ± 0.09
C 25:2 Δ 5,9	3.01 ± 0.52	3.91 ± 0.21	3.11 ± 0.12	3.25 ± 0.06
ΣPUFA	24.83 ± 2.12	24.16 ± 4.12	22.58 ± 1.12	22.5 ± 3.32
	OBFA	OBFA	OBFA	OBFA
Anteis o –C 15:0	0.52 ± 0.12	0.88 ± 0.07	1.62 ± 0.12	2.0 ± 0.11
C 15:0	6.53 ± 0.65	7.03 ± 0.35	6.13 ± 0.55	6.51 ± 0.33
Iso- C16:0	–	0.45 ± 0.01	1.62 ± 0.14	1.66 ± 0.09
Anteis o –C 17:0	2.02 ± 1.1	2.12 ± 0.1	3.12 ± 1.1	4.22 ± 1.46
Iso -C 17:0	0.52 ± 0.11	0.81± 0.1	0.72 ± 0.01	0.88 ± 0.1
Iso -C 17:1(n-5)	0.75 ± 0.12	0.81 ± 0.08	0.95 ± 0.02	1.1 ± 0.25
C 17:0	6.98 ± 0.55	6.85 ± 0.06	5.18 ± 0.25	5.72 ± 0.6
Iso -C 18:0	1.09 ± 0.13	1.49 ± 0.23	0.78 ± 0.11	1.06 ± 0.09
14-methyl-C 18:0	2.55 ± 0.12	2.33 ± 0.09	2.15 ± 0.32	2.34 ± 0.01
Anteis o –C 18:0	1.02 ± 0.2	1.91 ± 0.1	1.32 ± 0.22	2.57 ± 0.14
C 19:0	1.33 ± 0.36	1.93 ± 0.66	1.23 ± 0.45	1.15 ± 0.17
C 21:0	1.48 ± 0.11	1.38 ± 0.37	1.48 ± 0.01	1.05 ± 0.19
2-Octyl-cyclopropaneoctanoic acid	–	1.0 ± 0.17	–	1.22 ± 0.1
2-Hexyl-cyclopropaneoctanoic acid	–	1.1 ± 0.32	–	1.18 ± 0.2
ΣOBFA	24.79 ± 2.03	29.64 ± 4.23	26.64 ± 2.03	30.66 ± 5.85

*Means are the averages of 3 replicates. The values are shown as mean ± standard deviation (SD).

and *Geloina coaxans* (Bachok *et al.,* 2003) and some deposit-feeding marine invertebrates (Meziane & Tsuchiya, 2000).

The identification of cyclopropane ring is an interesting finding of the fatty acids components in the studied nudibranchs. These fatty acids have been found in bacteria, parasites, ascidia, sponges and plants (Carballeira *et al.,* 2007; Rob *et al.,* 2011; Yu *et al.,* 2011). Cyclopropane fatty acids contain three carbocyclic rings and it can be located at various position of fatty acids chain. Cyclopropaneoctanoic acid 2-hexyl fatty acid has been characterized in *Erythrobacter* sp. strains isolated from the upper ocean (Koblizek *et al.,* 2003). The two fatty 2-octyl-cyclopropaneoctanoic acid and 2-hexyl-cyclopropaneoctanoic acid have been detected in human adipose tissue (Sledzinski *et al.,* 2013). These fatty acids were detected in the notum of *P. varicosa* and *P. pustulosa* with small concentrations.

It is uncertain that a wide variety of odd- branched chain fatty acids were detected in the two sea slugs seems to be originated from dietary sources. Therefore, it may presume that these fatty acids may have come from symbiotic bacteria living in the host tissue or from bacteria in the food chain. The symbiotic bacteria may provide different functions for the host tissue of *D. nigra*, such as involvement in defense from predators and nutritional role in the nudibranch (Zhukova & Eliseikina, 2012). The occurrence and distribution of OBFA dominated by C15:0 and C17:0 in exceptional amounts may serve as evidence that these symbiotic bacteria provide the sea slugs with nutrients. The concentrations of iso-C15:0, anteiso-C15:0, iso-C16:0, C15:0, iso-C17:0, anteiso-C17:0, C17:0, iso-C18:0 and anteiso-C18:0 acid in the notum were higher than same fatty acids in viscera and this supports our hypothesis. The *Polychaete osedax* sp. inhabit the whalebone and host microflora, and the results of this association showed that the bacterial fatty acids composition in the worm obtains from the symbiotic bacteria (Goffredi *et al.,* 2005). The proportions of OBFA in notum of *P. varicosa* and *P. pustulosa* were so elevated that it seems unlikely that sponges are the main source of bacteria for the sea slug. The concentrations of OBFA in the notum of two sea slugs were higher than the viscera and this indicate bacterial sources may originate this variation.

The two sea slugs showed unique features in their fatty acid composition. They displayed high abundance of OBFA, and various MUFA and PUFA. These fatty acids originate from De novo synthesis and symbiotic partnerships with bacteria. The findings of the current study and previous research suggested that symbiotic bacteria may play an essential role in producing OBFA. This study has shown that these sea slugs may serve as a host for symbiotic bacteria.

ACKNOWLEDGMENTS

The authors are grateful to the staff at Institute of Oceanography and Maritime Studies (INOCEM) - IIUM for providing the diving equipment's. We wish to thank the scuba diving team for their valuable assistance in sample collection. This project was funded by e-science fund with grant number SS12-016-0045.

REFERENCES

Bachok, Z., Mfilinge, P.L. & Tsuchiya, M. 2003. The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. *Journal of Experimental Marine Biology and Ecology,* **292**(2): 187-197.

Brunckhorst, D.J. 1993. The systematics and phylogeny of phyllidiid nudibranchs (Doridoidea). 1st Ed., Sydney. 15pp.

Carballeira, N.M., Montano, N., Vicente, J. & Rodriguez, A.D. 2007. Novel cyclopropane fatty acids from the phospholipids of the Caribbean sponge *Pseudospongosorites suberitoides*. *Lipids,* **42**(6): 519-524.

Dalsgaard, J., John, M.S., Kattner, G., Müller, N.D., & Hagen, W. 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology,* **46**: 225-340.

Fallick, A.E. & Sargent, J.R. 1997. Lipid and lipid carbon stable isotope composition of the hydrothermal vent shrimp *Mirocaris fortunata*: evidence for nutritional dependence on photosynthetically fixed carbon. *Marine Ecology Progress Series,* **157**: 221-231.

Gillan, F. & Johns, R.B. 1986. Chemical markers for marine bacteria: fatty acids and pigments. *Methods in Geochemistry and Geophysics,* **24**: 291-309.

Gillan, F., Stoilov, I.L., Thompson, J.E., Hogg, R.W., Wilkinson, C.R. & Djerassi, C. 1988. Fatty acids as biological markers for bacterial symbionts in sponges. *Lipids,* **23**(12): 1139-1145.

Goffredi, S.K., Orphan, V.J., Rouse, G.W., Jahnke, L., Embaye, T., Turk, K. & Vrijenhoek, R.C. 2005. Evolutionary innovation: a boneeating marine symbiosis. *Environmental Microbiology,* **7**(9): 1369-1378.

Ichihara, K.I. and Y. Fukubayashi. 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research,* **51**(3): p. 635-640.

Kamenev, G.M. 1995. Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). *Marine Ecology Progress Series,* **120**: 231-241.

Kandhro, A., Sherazi, S.T.H., Mahesar, S.A., Bhanger, M.I., Talpur, M.Y. & Latif, Y. 2010. Variation in fatty acids composition including trans fat in different brands of potato chips by GC-MS. Pakistan *Journal of Analytical and Environmental Chemistry,* **1**: 36-41.

Kaneda, T. 1991. Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiological Reviews,* **55**(2): 288-302.

Klussmann-Kolb, A. & Brodie, G.D. 1999. Internal storage and production of symbiotic bacteria in the reproductive system of a tropical marine gastropod. *Marine Biology,* **133**(3): 443-447.

Koblizek, M., Béjà, O., Bidigare, R.R., Christensen, S., Benitez, N.B., Vetriani, C. & Kolber, Z.S. 2003. Isolation and characterization of *Erythrobacter* sp. strains from the upper ocean. *Archives of Microbiology,* **180**(5): 327-338.

McKenzie, J.D., Black, K.D., Kelly, M.S., Newton, L.C., Handley, L.L., Scrimgeour, C.M. & Henderson, R.J. 2000. Comparisons of fatty acid and stable isotope ratios in symbiotic and non-symbiotic brittlestars from Oban Bay, Scotland. *Journal of the Marine Biological Association of the UK,* **80**(2): 311-320.

Meziane, T. & Tsuchiya, M. 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. *Marine Ecology Progress Series*. **200**: p. 49-57.

Perry, G.J., Volkman, J.K., Johns, R.B. & Bavor, H.J. 1979. Fatty acids of bacterial origin in contemporary marine sediments. *Geochimica et Cosmochimica Acta,* **43**(11): 1715-1725.

Pond, D.W., Allen, C.E., Bell, M.V., Van Dover, C.L., Fallick, A.E., Dixon, D.R. & Sargent, J.R. 2002. Origins of long-chain polyunsaturated fatty acids in the hydrothermal vent worms *Ridgea piscesae* and *Protis hydrothermica*. *Marine Ecology Progress Series,* **225**: 219-226.

Pranal, V., Fiala-Medioni, A. & Guezennec, J. 1997. Fatty acid characteristics in two symbiont-bearing mussels from deep-sea hydrothermal vents of the south-western Pacific. *Journal of the Marine Biological Association of the United Kingdom,* **77**(2): 473-492.

Püttmann, M., Krug, H., von Ochsenstein, E. & Kattermann, R. 1993. Fast HPLC determination of serum free fatty acids in the picomole range. *Clinical Chemistry,* **39**(5): 825-832.

Rob, T., Ogi, T., Maarisit, W., Taira, J. & Ueda, K. 2011. Isolation of C11 Compounds and a Cyclopropane Fatty Acid from an *Okinawan Ascidian, Diplosoma* sp. *Molecules,* **16**(12): 9972-9982.

Saito, H. & Hashimoto, J. 2010. Characteristics of the fatty acid composition of a deep-sea vent gastropod, *Ifremeria nautilei. Lipids,* **45**(6): 537-548.

Sledzinski, T., Mika, A., Stepnowski, P., Proczko-Markuszewska, M., Kaska, L., Stefaniak, J. & Swierczynski, J. 2013. Identification of cyclopropaneoctanoic acid 2-hexyl in human adipose tissue and serum. *Lipids,* **48**(8): 839-848.

Stowasser, G., Pond, D.W. & Collins, M.A. 2012. Fatty acid trophic markers elucidate resource partitioning within the demersal fish community of South Georgia and Shag Rocks (Southern Ocean). *Marine Biology,* **159**(10): 2299-2310.

Wright, A.D. 2003. GC-MS and NMR analysis of *Phyllidiella pustulosa* and one of its dietary sources, the sponge Phakelliacarduus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **134**(2): p. 307-313.

Yasman, E., Ru A., Wray, V. & Proksch, P. 2003. New 9-Thiocyanatopupukeanane Sesquiterpenes from the Nudibranch Phyllidia varicosa and Its Sponge-Prey Axinyssa a culeata. *Journal of Natural Products,* **66**(11): 1512-1514.

Yu, X.H., Rawat, R. & Shanklin, J. 2011. Characterization and analysis of the cotton cyclopropane fatty acid synthase family and their contribution to cyclopropane fatty acid synthesis. *BMC Plant Biology,* **11**(1): 97.

Zhukova, Natalia V. 2007. Lipid classes and fatty acid composition of the tropical nudibranch mollusks *Chromodoris* sp. and *Phyllidia coelestis. Lipids,* **42**(12): 1169-1175.

Zhukova, N.V. 2014. Lipids and fatty acids of nudibranch mollusks: Potential sources of bioactive compounds. *Marine Drugs,* **12**(8): p. 4578-4592.

Zhukova, N.V. & Eliseikina, M.G. 2012. Symbiotic bacteria in the nudibranch mollusk *Dendrodoris nigra*: fatty acid composition and ultrastructure analysis. *Marine Biology,* **159**(8): 1783-1794.

Zhukova, N.V., Kharlamenko, V.I., Svetashev, V.I., & Rodionov, I.A. 1992. Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. *Journal of Experimental Marine Biology and Ecology,* **162**(2): 253-263.