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Fatty Acid Profiles of Marine Benthic Microorganisms Isolated from the Continental Slope of Bay of Bengal: A Possible Implications in the Benthic Food Web

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Abstract – Marine bacteria, actinomycetes and fungal strains were isolated from continental slope sediment of the Bay of Bengal and studied for fatty acid profile to investigate their involvement in the benthic food-web. Fifteen different saturated and unsaturated fatty acids from bacterial isolates, 14 from actinomycetes and fungal isolates were detected. The total unsaturated fatty acids in bacterial isolates ranged from 11.85 to 37.26%, while the saturated fatty acid ranged between 42.34 and 80.74%. In actinomycetes isolates, total unsaturated fatty acids varied from 27.86 to 38.85% and saturated fatty acids ranged from 35.29 to 51.25%. In fungal isolates unsaturated fatty acids ranged between 44.62 and 65.52% while saturated FA ranged from 20.80 to 46.30%. The higher percentages of unsaturated fatty acids from the microbial isolates are helpful in anticipating the active participation in the benthic food-web of Bay of Bengal.

Key words – marine bacteria, actinomycetes, fungi, fatty acid profile, Bay of Bengal

1. Introduction

Besides the role of microorganisms as potential decomposers in the microbial loop in the marine environment, microorganisms provide essential nutrients in marine food webs in the form of B-complex vitamins (Phillips 1984) and as a food source in aquaculture (Intriago and Jones 1993). Bacteria supply essential nutrients directly as a primary food source for omnivorous and filtering benthic animals (Sorokin 1993) and the commensal microbial communities of marine animals (Nichols 2003). Herbivorous marine fishes digest the dietary fibre compounds through short chain fatty acids, CO_2, H_2, and CH_4 (Stevens 1988). Microbial fatty acids are also significant in the detritivorous diet. Further, marine microorganisms are the anticipated sources of unusual novel lipids (Russell and Nichols 1999).

Many marine organisms lack the *de novo* ability to produce PUFA and hence rely on a dietary supply of n-3 PUFA such as eicosapentaenoic acid [EPA; 20: 5(n-)3], docosahexaenoic acid [DHA; 22: 6(n-)3] or closely related C18 precursors (Kanazawa *et al.* 1979; Intriago and Jones 1993). The effect of this dependence has been demonstrated in a recent study where the level of EPA in the food web was of central importance in controlling the efficiency of energy and biomass transfer at the pelagic producer-consumer interface (Muller-Navarra *et al.* 2000). Microalgae have long been considered the major *de novo* producer of PUFA in marine food webs (Gonzalezbaro and Pollero 1988) although further sources such as the lipid-rich thraustochytrids have been suggested as making an appreciable contribution (Lewis *et al.* 1999).

The ability of marine bacteria to produce PUFA is now also established (Russell and Nichols 1999; reviewed by Nichols and McMeekin (2002). Their presence among prokaryotes was often shown in deep-sea bacteria (DeLong and Yayanos 1986; Hamamoto *et al.* 1994). Besides the biochemical function of PUFAs, they are also interesting in relation to ecological aspects of the food web in the deep sea (Hamamoto *et al.* 1994). It has also been stated that while PUFA production appears as a phylogenetically linked genotypic strategy for such selective pressures, their presence

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may not be essential for the growth of bacteria in the environment (Nichols 2003).

Fatty acid profiles of obligate marine fungi were studied and reported to contain higher amount of PUFA (Block *et al.* 1973; Cooney *et al.* 1993). Shirasaka *et al.* (1995) reported the occurrence of a furan fatty acid from commonly occurring marine bacteria isolated from fish intestine. The cellular fatty acid compositions of bacterial isolates have been studied (Hamamoto *et al.* 1995; Yazawa 1996; Watanabe *et al.* 1996; Cho and Mo 1999; Yanagibayashi *et al.* 1999; Kato 1999; De Rosa *et al.* 2000; Skerratt *et al.* 2002; Ivanova *et al.* 2004; Lee *et al.* 2006).

Fang *et al.* (2002) studied the fatty acid composition of deep sea bacteria from the Mariana Trench. Nichols and McMeekin (2002) summarized the current biodiversity of PUFA- producing bacterial genera. Pujari *et al.* (2004) and Devi *et al.* (2006) reported the fatty acid composition of marine bacteria and marine fungi respectively.

However, microbial isolates from the Bay of Bengal have not been investigated for their fatty acid profile so far. Therefore, the present study aims to determine the fatty acid profiles of marine microorganisms isolated from the sediments of the continental slope of the Bay of Bengal, and, further, to use the findings to predict the involvement of these strains in the benthic food web.

2. Materials and Methods

The sediment samples were collected using Smith McIntyre Grab during Cruise No. 225 and 236 of FORV *Sagar Sampada* in 2004 and 2005 respectively, covering 33 stations over 11 transects in Bay of Bengal (lat. 10°36'N to 20°01'N and long. 79°59'E to 87°30'E) as described elsewhere (Das *et al.*, 2007a). Surface plating for cultivable fraction of bacteria was carried out on board immediately after collection of sediment samples onto Marine Agar 2216e medium (HiMedia, India) in duplicate by spread plate method after suitable dilutions (10^{-3} and 10^{-4}). Actinomycetes and fungal colonies were isolated onto Starch-casein agar (HiMedia, India) and Rose Bengal agar (HiMedia, India) media respectively. The plates were then incubated for 2 days, 15 days and 7 days at 25 °C respectively to count the colony forming units. The microbial isolates were then stored in 5 °C until further study.

Randomly isolated colonies were picked up for the fatty acid analysis. The generic level identification of bacterial isolates was done according to the methods described in Das *et al.* (2007b, 2007c). Actinomycetes is also a kind of bacteria (Gram positive high G+C group), and the isolates were identified by classical chemotaxonomy following Lechevalier and Lechevalier (1970) since the GC result by MIDI software could not provide the information about these marine microorganisms. Fungal isolates were identified by the taxonomic keys given by Kohlmeyer and Kohlmeyer (1991).

Microbial strains were prepared for fatty acid methyl ester (FAME) analysis by gas chromatography following the method of Sasser *et al.* (2005). The reagents and procedure were as follows:

Preparation of Reagents

***Reagent I*:** 45g NaOH, 150 ml methanol (LC grade) and 150 ml deionised water.

Reagent II: 325 ml 6 N hydrochloric acid and 275 ml methanol (LC grade).

Reagent III: 200 ml hexane (LC grade) and 200 ml Methyl-ter-butyl ether (MTBE).

Reagent IV: 10.8 g NaOH was dissolved in 900 ml deionised water.

Saturated NaCl: 40 g NaCl was dissolved in 100 ml deionised water.

Preparation of Samples

***Bacterial samples*:** From the third quadrant of the pure culture of bacterial isolates, approximately 40 mg cells (one heaping 4 mm loopful) were transferred onto the lower inner surface of the screw cap tubes.

***Actinomycetes and fungal samples*:** The actinomycete and fungal isolates were grown in 500 ml Erlenmeyer flasks containing Yeast Extract-Malt extract and 2% malt extract broth respectively, prepared with aged seawater (pH 8.0) at 24±2 °C with constant shaking. Mycelia were harvested from exponential cultures by centrifugation. The mycelial pellets were washed with distilled water in screw cap tubes. Further preparation was as follows:

***Saponification*:** 1.0±0.1 ml Reagent I, the methanolic base, was transferred into each of the culture tubes in a water bath. After a total of 30 min of saponification in water bath (80°±1 °C), tubes were cooled immediately.

***Methylation*:** 2.0±0.1 ml Reagent II, the methylation reagent, was added to each tube. The solution was mixed by a vortex mixer for 5-10 seconds. Then the tubes were placed in a water bath (80°±1 °C) for 10±1 min.

Extraction: 1.25±0.1 ml Reagent III, the extraction solvent, was added to each tube. The tubes were then placed in laboratory rotator and gently mixed end-over-end for 10±1 min. The aqueous (lower) phase was removed by using a clean pipette for each sample.

Base wash: 3.0±0.1 ml Reagent IV, the base wash, was added to each tube. The tubes were rotated gently end-over-end for 5 min. Then a few drops of saturated NaCl water solution were added to the tube to aid in breaking the emulsion.

Transfer of extract to sample vial: Using a clean pipette for each sample, about 2/3rd of the organic (upper) phase from the tube was transferred to a clean GC sample vial.

The purified methyl esters were analyzed by a gas chromatograph (Agilent- GC 6890N) equipped with flame ionization detector. Capillary column- HP Ultra 2 with 2 m long and 0.2 mm i.d. (inner diameter), coated with 5% phenyl methyl silaxane and 0.33 μm thickness was used. The rate of hydrogen carrier gas flow was maintained at 30 ml/min. FAMEs were identified by MIDI calibration standard software.

3. Results

Twenty bacterial isolates, eight actinomycete isolates and twelve fungal isolates were randomly selected from both the cruises for fatty acid analysis. The identification of fatty acids and their percentage composition are presented in Tables 1 and 2.

Fatty acids of bacteria: Fifteen different saturated and unsaturated fatty acids were derived from the twenty bacterial isolates. The number and percentage of fatty acids in each species varied considerably. The detected fatty acids ranged from 12 to 20 carbons. The average major percentage was contributed by 16:0 and 18:0 followed by 15:0 and the least was contributed by 20:1n7, which was found in only 5 bacterial isolates. The total unsaturated fatty acids varied considerably, ranging from 11.85 to 37.26%, while the saturated fatty acid ranged between 42.34 and 80.74%.

Fatty acids of actinomycetes and fungi: Fourteen different saturated and unsaturated fatty acids were detected from actinomycete and fungal isolates. In each organism the fatty acids detected ranged from 12 to 22 carbons. Linoleic (18:2n6), palmitic (16:0) and oleic (18:1n9) acids were the principal fatty acids found in actinomycete and fungal isolates. The total unsaturated fatty acids varied from 27.86 to 38.85% in actinomycetes and 44.62 to 65.52% in fungi. Saturated FA ranged from 34.29 to 51.25% and 20.80 to 46.30% in actinomycetes and fungal isolates respectively.

4. Discussion

The microbial isolates from the continental slope of the Bay of Bengal yielded a total of 15 FA (15 from bacteria and 14 from actinomycete and fungi). Nadimuthu (1998) reported nine different saturated and unsaturated fatty acids from marine fungi. Pujari *et al.* (2004) reported 12 fatty acids from marine bacteria. Cooney *et al.* (1993) reported 13 fatty acids from marine fungi. Devi *et al.* (2006) reported 10 fatty acids from marine fungi. Therefore, in the present study, more numbers of fatty acids were detected, which provides information about the possible enrichment of the dietary budget of the marine deposit feeders in this region.

PUFA are essential dietary factors in both terrestrial and aquatic animals. A deficiency of n6 and n3 PUFA in the diet causes definite symptoms such as depigmentation, fin and skin erosion and shock syndrome in fishes (Sargent 1976). Fishes cannot accomplish *de novo* synthesis of these polyunsaturated fatty acids (Steffens and Wirth 1997). However, fish can desaturate or elongate the other dietary fatty acids like 18: 2, which was identified in the present study. In addition, the FA 16: 0 derived from the microorganisms in the present study has been reported as a predominant fatty acid component in many marine fishes (Sargent 1976). Fatty acids such as 12:0, 15:0, 17:0 and 18: 1n7 along with the FA 16: 1n7 obtained in the present study are known for their structural role in the embryogenesis of bivalves (Whyte 1988; Whyte *et al.* 1990).

Apart from self synthesis of fatty acids, consumers of different trophic levels obtain fatty acids from their food sources. Based on the feeding habits of the organisms, the dietary fatty acid source to the consumers may vary. While carnivores obtain fatty acids from the living animals of the environment in which they feed, the omnivores get them partially and the herbivores get them fully from plant materials.

The bulk of *de novo* biosynthesis of the two long-chained PUFA of the n-3 series (20:5 n-3; EPA and 22:6 n-3; DHA) is thought to take place among phototrophic algae at the base of the marine food web. From there, the PUFA are

Table 1. Fatty acid profile (relative % composition) of bacterial isolates

Fatty acids	Bacterial isolates																		
	DSB 225.22 (*Pseudomonas*)	DSB 225.63 (*Vibrio*)	DSB 225.107 (*Pseudomonas*)	DSB 225.182 (*Vibrio*)	DSB 225.225 (*Flavobacterium*)	DSB 225.253 (*Alteromonas*)	DSB 225.335 (*Bacillus*)	DSB 225.419 (*Flavobacterium*)	DSB 225.440 (*Vibrio*)	DSB 225.498 (*Bacillus*)	DSB 225.514 (*Bacillus*)	DSB 236.42 (*Bacillus*)	DSB 236.75 (*Vibrio*)	DSB 236.167 (*Pseudomonas*)	DSB 236.298 (*Pseudomonas*)	DSB 236.324 (*Cytophaga*)	DSB 236.404 (*Bacillus*)	DSB 236.440 (*Alteromonas*)	DSB 236.465 (*Bacillus*)	DSB 236.515 (*Alteromonas*)
12:0	3.15	3.38	4.23	9.92	3.48	6.68	3.43	2.46	0.37	9.62	-	2.86	0.88	7.86	-	4.23	-	4.48	5.23	-
14:0	7.69	2.71	4.43	-	15.11	3.04	2.68	7.26	6.28	3.44	10.09	8.14	1.33	6.59	5.88	8.22	8.98	-	5.93	6.59
15:0	15.57	8.66	3.55	3.64	8.59	-	-	14.02	3.92	3.31	34.49	3.52	5.32	6.36	10.96	14.64	9.43	12.78	6.33	12.87
15:1	5.73	3.55	6.58	-	2.24	1.56	1.62	6.71	1.03	7.47	31.65	3.49	2.76	1.30	-	-	1.66	5.87	-	0.79
16:0	15.62	19.58	29.43	-	0.75	30.83	26.08	0.58	16.10	23.09	-	33.57	14.73	26.22	10.85	10.92	14.13	16.59	14.32	10.10
16:1n7	1.99	3.70	2.76	22.92	8.87	1.09	3.32	4.55	4.12	4.51	-	6.99	6.40	6.69	5.83	9.28	13.07	15.95	8.57	-
17:0	12.02	9.45	3.12	-	27.34	5.68	3.76	11.72	34.33	5.46	23.77	4.89	8.45	1.16	10.91	9.93	11.91	15.17	18.31	16.33
18:0	12.52	28.99	25.75	52.23	19.53	13.52	21.09	21.53	12.89	19.09	-	6.15	7.08	7.38	13.16	16.84	5.81	12.67	-	18.65
18:1n9	8.92	1.93	5.90	-	5.12	3.83	5.98	4.01	3.84	6.10	-	4.98	8.83	5.67	-	2.56	-	-	6.58	7.12
18:1n7	2.00	-	3.65	-	3.10	14.70	18.64	2.79	1.51	-	-	7.28	17.12	4.04	7.72	5.72	2.17	6.70	3.62	4.19
18:3n3	2.55	-	-	-	-	0.98	1.33	3.03	0.84	2.43	-	0.28	-	-	1.45	4.18	3.50	1.13	0.54	1.35
19:0	2.00	3.53	2.67	-	2.05	6.80	5.61	6.47	6.85	4.96	-	3.29	3.43	2.82	-	1.05	-	-	-	5.05
19:1	3.63	2.67	-	3.74	-	3.62	2.74	2.15	1.25	6.31	-	4.82	2.15	-	-	-	-	2.41	1.23	0.69
20:0	-	-	1.16	-	2.01	-	-	-	-	-	-	0.19	1.12	0.59	-	-	8.25	-	2.15	-
20:1n7	0.64	-	-	-	-	-	-	-	-	-	-	1.07	-	-	0.50	-	0.61	0.57	-	-
Total unsaturated	25.46	11.85	18.89	26.66	19.33	25.78	33.63	23.24	12.59	24.39	31.65	28.91	37.26	17.70	14.05	21.74	21.01	32.63	20.54	14.14
Total saturated	68.57	76.30	74.34	65.79	78.86	66.55	62.65	64.04	80.74	71.40	68.35	62.61	42.34	58.98	53.21	65.83	58.51	61.69	52.27	69.59

*The nomenclature of fatty acids is as follows- the number of C atoms in the fatty acid is indicated by the number before the colon and the number after the colon indicates the number of double bonds. 'n' indicates the position of the first double bond from the aliphatic or omega end of the molecules.

Table 2. Fatty acid profile (relative % composition) of actinomycetes and fungal isolates

Fatty acids	Actinomycetes and fungal isolates																			
	DSA 225.06 (*Streptomyces*)	DSA 225.19 (*Streptomyces*)	DSA 225.48 (*Streptomyces*)	DSA 225.52 (*Streptomyces*)	DSA 225.64 (*Streptomyces*)	DSA 236.60 (*Streptomyces*)	DSA 236.88 (*Streptomyces*)	DSA 236.91 (*Streptomyces*)	DSF 225.43 (*Aspergillus niger*)	DSF 225.44b (*Penicillium chrysogenum*)	DSF 225.47b (*Corollospora maritima*)	DSF 225.55 (*Paecilomyces humicola*)	DSF 225.64b (*Aspergillus versicolor*)	DSF 225.68 (*Aspergillus* sp.)	DSF 225.84 (*Lulworthia* sp.)	DSF 236.89a (*Aspergillus flavus*)	DSF 236.101 (*Penicillium* sp.)	DSF 236.117 (*Lulworthia* sp.)	DSF 236.144 (*Tricoderma* sp.)	DSF 236.150a (*Alternaria maritima*)
12:0	0.80	0.70	1.02	-	0.50	-	0.65	1.23	1.33	1.30	0.30	-	-	0.20	-	0.25	-	0.50	0.05	0.50
14:0	1.50	2.50	0.85	1.44	2.50	-	-	0.87	-	2.56	0.60	1.90	1.05	0.30	0.30	2.30	1.25	1.30	0.26	1.67
16:0	17.60	12.34	16.45	15.65	19.57	25.48	18.65	20.50	35.58	15.66	21.60	25.64	18.55	20.12	15.40	20.50	18.66	18.56	18.54	12.51
16:1n7	15.20	12.50	11.46	8.37	10.44	7.16	12.55	8.54	-	12.20	-	2.60	1.35	0.55	1.00	2.50	10.47	1.50	0.55	3.51
17:0	0.60	1.60	0.55	2.35	5.05	1.55	2.46	5.30	3.65	5.68	0.70	1.50	0.85	1.05	0.60	1.55	6.50	-	-	0.70
18:0	15.30	16.55	28.34	20.69	19.78	18.47	25.54	18.74	5.24	7.53	3.20	-	15.54	10.58	4.20	18.65	4.12	5.60	8.55	8.13
18:1n9	-	-	0.56	-	0.50	-	-	1.60	25.84	28.75	22.30	29.88	28.56	23.58	24.10	32.56	19.64	25.54	38.25	25.64
18:1n7	-	0.50	0.18	-	1.25	-	3.50	-	1.55	1.05	0.60	-	0.52	5.23	0.60	2.33	2.35	-	0.30	0.53
18:2n6	22.55	18.74	15.35	22.50	19.55	28.94	19.56	19.34	12.38	15.54	35.20	24.00	18.89	21.40	35.30	11.20	13.65	37.33	22.78	15.42
18:3n3	1.10	0.55	-	-	1.52	-	0.50	-	4.50	-	1.10	6.45	5.63	3.33	1.40	1.50	-	0.50	0.58	-
20:0	0.50	0.60	2.54	1.25	-	2.40	3.65	3.54	0.50	-	0.20	-	0.80	0.65	0.20	-	0.80		1.20	2.36
20:1n9	-	0.20	-	-	-	-	-	2.70	-	3.57	0.30	-	-	0.10	0.10	-	1.23	0.15	-	-
20:4n6	-	-	0.31	-	0.60	0.80	-	1.50	0.35	-	-	0.58	-	0.30	0.20	0.50	0.50	0.50	-	5.11
22:0	0.30	-	0.25	0.50	-	-	0.30	-	-	0.50	0.10	0.10	0.10	0.15	0.10	-	-	-	0.55	1.30
Total unsaturated	38.85	32.49	27.86	30.87	33.86	36.90	36.11	33.68	44.62	61.11	59.50	63.51	54.95	54.49	62.70	50.59	47.84	65.52	62.46	50.21
Total saturated	38.60	34.29	50.00	41.88	47.40	47.90	51.25	50.18	46.30	33.23	26.70	29.14	36.89	33.05	20.80	43.25	31.33	25.96	29.15	27.17

transferred through trophic levels and accumulate as major constituents of the lipids of practically all marine animals. In the present study, C18 polyunsaturates were found in the microorganisms. The presence of n-3 PUFA in heterotrophic prokaryotes was first reported by Johns and Perry (1977) in the marine bacterium *Flexibacter polymorphus*. Later, DeLong and Yayanos (1986) reported PUFAs in deep-sea barophiles and *Vibrio marinus* MP-1. Jostensen and Landfald (1997) also reported PUFA from several marine bacteria. Similar PUFA (20:5) were also found in Antarctic bacteria (Nichols *et al.* 1993).

During the decomposition process in the sediment, the original fatty acids of plant materials are converted into microbial fatty acids (of the decomposers *viz.* bacteria and fungi) in accordance with different stages of decomposition and association of microbial colonizers at each stage (Findlay *et al.* 1986). Thus, the essential fatty acids in the dietary budget of herbivores and omnivores are derived from the microbial fatty acids of the food-web process.

The predominate fatty acid in the marine fishes was reported to be palmitic acid (16:0) and it was suggested that the differences in fatty acid composition among various marine fish species may be due to differences in diet (Tanakol *et al.* 1999; Visentainer *et al.* 2007). Therefore, the diet (taking for granted it contains a substantial amount of microorganisms) may provide the fatty acids which are also predominant in the microbial isolates in the present study in Bay of Bengal.

Branched-chain fatty acids have been considered as a biomarkers for actinomycete and gram positive bacteria in natural samples (Zelles and Bai 1994) and the fatty acids present in the microorganisms are considered unique to be biomarker fatty acids (Zelles *et al.* 1995). Therefore, the fatty acid profiles are useful tools in systematics- notwithstanding the complications associated with the fact that fatty acid composition is sensitive to external factors like temperature, medium, and phase of growth (Jostensen and Landfald 1996). Therefore, the fatty acid profiles of the present study agrees well with those reported for Pseudomonadaceae and Vibrionaceae (Wilkinson 1988).

This study has demonstrated the presence of fifteen different saturated and unsaturated essential dietary fatty acids in the common bacteria, actinomycete and fungi of the Bay of Bengal, all of which are an important source of fatty acids to bottom feeding animals.

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