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Screening of bacteria from sediments of coastal ecosystem, as potential sources of alpha linolenic acid

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Marine bacteria, known to produce wide range of molecules that are beneficial to animals as well as to human beings, were screened for the presence of alpha linolenic acid (9,12,15-octadeca trienioc acid). The lipid and protein concentrations of predominant bacterial isolates, obtained from coastal marine sediment were determined. Out of twenty isolates, eight bacterial isolates with higher lipid – protein ratio (more than 0.5), were grown in mineral salt medium with sodium acetate as carbon source as well as in nutrient broth. Their lipid (triglyceride, sterol, fatty acid, glycolipid and phospholipid) and fatty acid (mainly C-18 series) profiles were analyzed. Only four bacterial isolates depicted significant conversion efficacy for alpha linolenic acid (more than 25%) when they were grown in sodium acetate media. Such bacteria can be used as supplement to enrich the animal feed with the required fatty acid.

[Key words: Linolenic acid, bacteria, sediment]

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Introduction

Polyunsaturated fatty acid act as precursor molecules for many biological compounds for e.g., prostaglandin, thromboxane etc., which help the organisms to remain in highly active and healthy condition[1]. However, animal systems do not synthesis PUFA directly from the dietary carbon source. The precursor molecules of PUFA viz. linoleic (9,12-octadeca dienoic) acid and alpha linolenic (9, 12,15-octadeca trienioc) acid are not been synthesized by animals, as they lack enzymes which could desaturase the oleic (9-octadeca monoenoic) acid. Hence, both linoleic and linolenic acid are known as essential fatty acids for animal system[2].

The concept of using microorganism in feed or enriching the feed with some specific microorganism in fish is well established in Asian countries. The use of living microbial supplementation in diet as an additional ingredient for enhancing growth of animal has been the thrust area of nutritionist in the recent past. These probiotics have multiple effects on intestinal micro flora and act as health promoting microorganisms [3].

Marine bacteria are known to produce wide range of compounds, which have potential applications as bioactive compounds, probiotics and nutritional supplements. These organisms are now being screened for the production of polyunsaturated fatty acids as well as specific fatty acids[4-6]. Since the natural distribution of alpha linolenic (9,12,15-octadeca trienoic) acid, over the linoleic (9,12-octadeca dienoic) acid, is very restricted, the present study was proposed to screen bacteria from marine ecosystems for potential production of alpha linolenic acid. We report here studies and characterization and lipid profiles of some bacterial isolates obtained from sediment samples from southwest coast of India.

Materials and Methods

Sediment samples were collected from Mangalore to Tuticorin (Table 1), south west coast of India at 50 m and 150 m contour depths during the cruise of *ORV Sagar Kanya* during October 2001. These samples were diluted with 0.85% saline and were plated on nutrient agar medium (NA). The predominant bacterial colonies were isolated, purified and stored on slants. The selected bacterial strains were grown in

mineral salt medium (MSM) containing 5% sodium acetate incubated on rotary shaker at room temperature for 48 hours. For comparison, same strains were grown in nutrient broth for 48 hours at room temperature. Cells were then harvested by centrifuging at 10,000 × *g* for 10 min. at 16°C, and washed with 0.85% saline. The bacterial pellet obtained was suspended in distilled water and sonicated. This suspension was used for extraction of bio-molecules. Lipid and protein were extracted from bacterial cell suspension by the method of Roy and Farkas [7]. The concentrated lipid was stored in 1 m*M* BHT (solution prepared in benzene).

Different lipid fractions were isolated by thin layer chromatography. The total sterol (by ferric chloride acid reagent), total triacylglycerol (using chromotropic acid reagent), total sugar containing lipid (by anthrone reagent), total phospholipid (using ammonium molybdate reagent) , total and free fatty acid (by titration) were estimated as per routine analytical procedures[8]. Total protein content of the bacterial isolates was estimated following the method of Lowry *et al.*[9] .

Methyl esters of fatty acid from the total lipid were prepared by trans esterification in distilled methanol containing 5% HCl at 90°C for three and half-hours[10]. The purified methyl esters were analyzed by a gas chromatograph (Chemito – GC 8610) equipped with a flame ionization detector. A stainless steel column (2 m long and o.d. 3 mm) packed with 10% DEGS on 100-200 meshes was used. The column temperature was programmed[10]. The rate of nitrogen carrier flow was maintained at 17 ml/minute. Fatty acid methyl esters were identified by comparison with reference standards of known composition (obtained from M/s Sigma Chemicals Co., U.S.A.).

Results and Discussions

Only the predominant colonies were isolated and stored on NA slants. The physical parameter, with respect to temperature, salinity and oxygen, of the environment (water) from where the sediment samples were collected showed a wide variation in the dissolved oxygen content (Table 1) however, variation was not seen in the salinity. It was interesting to note that the sediment samples had high concentration of carbon, nitrate and phosphate in comparison to surrounding water (Table 1). The sediment samples collected during *ORV Sagar Kanya* cruise were plated on media and predominant organisms were isolated. The counts varied from 13×10^5 to 610×10^5 (Table 2). The correlation between nutrient concentration and number of bacterial colonies is well-documented[11]. The dynamics of sediments differ completely from water

Table 1—Physicochemical parameters of the environment (water) from where the sediment sample was collected during cruise

Sl. no.	Position		Contour depth(m)	Press. (paros)	Temp. (deg. C)	Salinity (‰)	Dissol. O_2 (ml/lit)	Nitrate (picog/l)	Carbon (μg/l)	Phosphate (picog g/l)
	Latitude	Longitude								
	Off Mangalore									
1	12 55.71N	74 25.09E	53	48	21.56	35.308	0.728	7.151	0.104	3.124
2	12 54.61N1	74 00.10E	152	150	16.97	35.126	1.148	7.032	0.135	4.025
	Off Calicut									
3	11 15.47N	75 09.74E	60	52	20.34	35.089	1.008	7.006	0.162	3.968
4	11 15.51N	74 55.63E	142	140	17.85	35.065	0.841	4.151	0.178	4.084
	Off Cochin									
5	09 58.12N	75 44.02E	63	52	21.68	35.077	0.868	7.564	0.104	3.711
6	09 56.34N	75 35.96E	150	141	16.41	35.083	0.561	4.657	0.150	4.388
	Off Trivandrum									
7	08 29.96N	76 34.58E	60	49	21.4	35.078	1.136	6.623	0.131	4.169
8	08 28.25N	76 25.64E	155	148	17.39	35.042	2.856	6.000	0.162	3.404
	Off Cape Comorin									
9	07 36.16N	77 37.15E	65	52	22.93	35.117	2.912	5.482	0.120	4.139
10	07 19.98N	77 33.82E	155	156	16.69	34.996	3.136	3.923	0.139	3.875
	Off Tuticorin									
11	08 37.62N	78 25.59E	60	54	26.4	35.2667	2.864	5.785	0.135	2.517
12	08 38.47N	78 28.08E	165	153	14.73	35.057	3.164	5.234	0.170	3.069

ecosystem as here large particulate matter settles and large number of adhered organisms play a role in the decomposition process[12]. The nutrients formed within the ecological niche thereby increase the productivity and nutritional levels. This is a direct effect on the total number of organisms as well as their physical and chemical composition.

Although large number of isolates were obtained from sediment samples, only few isolates had high concentration of total lipid and lipid protein ratio varied from 0.57 to 0.88 (Table 3). These isolates were selected for their lipid and fatty acid profiles. Metabolism in bacteria depends upon carbon sources supplied as growth nutrients, which also help in directing the desired accumulation of the metabolites [13]. Induction of oxidation pathways for lipid is found to be regulated with simple carbon sources such as acetate, citrate etc. The bacteria grown on MSM containing 5% sodium acetate as carbon source showed better growth of the bacteria with increased lipid concentration, as compared to the bacteria grown on nutrient broth (Table 4). Acetyl-CoA being the common precursors of the different lipid molecules (viz. fatty acid and sterol), the excess acetate molecules converted into acetyl CoA is then directed towards the different biosynthetic routes of lipid molecules[14]. The augmentation of the total fatty acids in different bacterial isolates, grown in sodium acetate, leads to change in the relative fatty acid profile of the isolates (Table 5).

Table 2—Nutrient analysis of sediment samples and number of bacterial colonoies

Sample no.	Depth (meter)	Nitrate (μg/l)	Carbon (mg/l))	Phosphate (μg/l))	Colonies (nos.×10^5/g sediment)
1A	53m	6.23	0.06	1.552	28.0
1B		9.89	0.073	3.174	13.0
2A	152m	7.12	0.045	1.422	14.0
2B		6.92	0.051	2.676	69.0
3A	60m	2.57	0.051	0.896	46.0
3B		1.28	0.075	0.588	78.0
4A	142m	4.74	0.08	1.294	130.0
4B		NS	NS	NS	NS
5A	63m	3.07	0.063	1.014	17.0
5B		NS	NS	NS	NS
6A	150m	15.03	0.042	2.796	143.0
6B		6.73	0.082	1.6	131.0
7A	60m	9.2	0.084	4.388	610.0
7B		2.67	0.056	1.462	145.0
8A	155m	3.76	0.062	1.482	29.0
8B		3.46	0.057	0.926	11.0
9A	65m	2.67	0.057	0.906	265.0
9B		NS	NS	NS	NS
10A	155m	2.87	0.057	0.586	173.0
10B		NS	NS	NS	NS
11A	60m	2.96	0.037	1.522	127.0
11B		5.54	0.096	1.732	64.0
12A	165m	5.54	0.097	3.294	17.0
12B		8.9	0.066	3.304	187.0

A—Top layer of the sediment
B—Bottom layer of the sediment
NS—No sample

Table 3—Percent yield value of total protein and total lipid in bacterial isolates obtained from sediment samples during cruise. Mean values of four estimation and their standard error were tabulated here.

Isolate No.	Protein (mg/ 100 mg of wet cells)	Lipid (mg/ 100 mg of wet cells)	Lipid/Protein* ratio
1A	8.14 ± 1.23	6.27 ± 0.72	0.77
1B	10.12 ± 1.87	8.96 ± 0.69	0.88
2A	9.27 ±1.01	3.14 ± 0.41	0.34
2B	7.16 ± 1.06	4.94 ± 0.42	0.67
3A	9.17 ± 1.38	2.76 ± 0.25	0.30
3B	6.16 ± 0.76	4.39 ± 0.37	0.71
4A	8.13 ± 1.2	4.66 ± 0.47	0.57
5A	9.46 ± 1.46	4.13 ± 0.38	0.44
6A	9.37 ± 1.26	6.39 ± 0.93	0.68
6B	10.36 ± 1.73	2.17 ± 0.22	0.20
7A	10.14 ± 1.65	3.72 ± 0.52	0.37
7B	8.16 ± 0.99	3.29 ± 0.32	0.40
8A	8.12 ± 0.78	6.18 ± 0.81	0.76
8B	7.12 ± 1.67	2.17 ± 0.27	0.30
9A	7.05 ± 1.23	3.09 ± 0.32	0.44
10A	8.16 ± 1.46	4.94 ± 0.43	0.60
11A	7.17 ± 0.98	3.14 ± 0.21	0.44
11B	8.72 ± 0.92	3.78 ± 0.25	0.43
12A	8.76 ± 1.02	3.16 ± 0.21	0.36
12B	8.16 ± 1.34	2.82 ± 0.24	0.34

*Calculated from mean values only
Note: Isolate no. 1A, 10A are Gram negative and rest are gram positive
Isolate no. 1B, 2B, 3A, 4A, 5A, 6B, 7B, 8A, 10A, 11B, 12B are cocci in shape
Isolate no. 1A, 2A, 3B, 6A, 7A, 8B, 9A, 11A, 12 A are rod in shape

It was interesting to note that, about 15% to 70% enhanced accumulation of total C-18 chain fatty acids in most of the organisms (except culture no. 2B, 3B, 4A and 10A) when grown in sodium acetate media as compared to nutrient broth. A 2 to 5 fold augmentation in the conversion efficacy of alpha linolenic acid (linolenic acid/total C-18×100) was noticed in bacterial isolates 1B, 2B, 4A and 8A and about 30% reduction in this conversion efficacy was noticed in the isolates 1A, 3B, 6A when these were grown in sodium acetate medium as compared to grown in nutrient broth media (Table 5). The ability to synthesis specific unsaturated fatty acid totally depends upon the carbon substrate used by the isolates. It is interesting to note that none of these isolates showed any traceable (more than 0.1%) amount of gama linolenic (6, 9, 12-octadeca trienioc) acid. Moreover, the metabolic fate of alpha linolenic acid and gama linolenic acid are quite different[1,15].

In order to select the best bacterial isolates amongst these for supplementing the feed, it was important to characterize the organisms so as to know their

Table 4—Comparative table showing the lipid profiles (n mol/mg protein) of bacteria grown on nutrient broth (NB) and sodium acetate (SA) media . Mean values of four replicate sets of experiment were tabulated

Lipid Profile	Culture no.															
	1A		1B		2B		3B		4A		6A		8A		10A	
	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA
TG	15.0	68.0	17.9	87.3	6.2	54.3	9.9	40.0	28.1	54.3	5.3	9.8	16.2	30.1	18.4	37.0
ST	0.41	1.9	1.2	8.2	0.09	5.2	1.6	5.2	1.3	2.7	1.2	1.2*	1.5	2.9	1.6	7.67
FFA	2.0	9.8	4.6	24.3	1.0	10.6	3.8	7.7	8.6	9.7*	7.3	7.5*	3.0	6.2	3.9	4.8*
GL	0.14	1.9	0.9	1.9	0.17	5.8	0.8	5.7	2.7	2.9*	0.45	0.9	0.4	0.5*	3.2	4.8
PL	8.6	27.9	16.0	54.9	3.1	27.0	7.9	21.5	9.7	21.2	20.9	38.7	10.2	18.2	21.5	47.0
TFA	74.3	292.9	111.2	417.9	26.0	233.0	53.2	192.1	115.1	217.4	68.3	121.5	72.0	143.7	103.5	215.0

NOTE: TG : Triacylglycerides; ST: Total sterol; FFA: Free fatty acid; GL: Glycolipid; PL: Phospholipid; TFA: Total fatty acid (free+ esterified); NB: nutrient broth media; SA: sodium acetate media.
*The changes are not statistically significant when the same was compared with the isolates grown on nutrient broth media.

Table 5—Comparative table showing the fatty acid profiles (relative % composition) of bacteria grown on nutrient broth (NB) and sodium acetate (SA) media. Mean values of four different replicate sets of experiment were tabulated.

Fatty acid profile	Culture no.															
	1A		1B		2B		3B		4A		6A		8A		10A	
	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA	NB	SA
Unknown	13.30	10.46	5.19	5.21	-	-	3.96	4.96	11.06	4.00	5.23	4.81	-	-	4.18	5.76
C-12:0	14.84	15.77	3.26	4.05	4.85	9.02	9.67	4.36	0.67	5.50	8.20	5.81	11.28	2.42	6.96	8.71
C-14:0	2.28	4.19	1.61	0.58	2.53	6.85	3.20	2.63	0.60	8.06	5.93	2.02	-	3.92	2.47	6.96
C-16:0	12.76	6.39	7.30	7.03	7.91	7.20	13.19	13.32	5.23	13.51	15.11	9.54	14.12	11.75	27.21	25.45
C-16:1	1.32	0.18	6.98	0.61	2.76	1.27	0.52	1.06	1.59	-	0.59	0.20	-	1.15	-	1.25
C-16:2	1.41	0.45	15.68	2.93	3.06	2.64	1.18	0.43	6.95	6.25	1.78	1.26	-	2.69	1.87	1.95
Unknown	-	-	5.68	-	-	-	2.69	-	0.65	-	0.60	3.72	10.57	-	1.97	0.74
C-18:0	1.71	0.40	16.96	22.63	7.54	10.98	2.24	2.36	2.15	2.26	1.89	3.52	1.61	2.07	5.87	0.74
C-18:1(CIS)	21.15	20.39	7.62	9.54	20.19	20.74	18.40	16.30	28.25	23.04	14.85	11.52	27.87	20.24	11.57	10.19
C-18:1(TR)	2.65	0.15	4.16	2.06	8.04	0.45	10.37	10.59	6.45	3.00	2.48	6.34	3.59	0.53	3.87	6.30
C-18:2(W6)	4.16	18.69	8.59	12.35	10.93	10.07	5.44	13.72	4.13	6.09	15.32	25.32	3.35	6.62	13.31	15.80
C-18:2(W3)	2.15	0.15	-	-	2.75	1.45	2.64	-	-	-	-	-	2.72	2.52	-	-
C-18:3(W3)	4.82	2.46	2.65	21.95	8.03	15.54	9.02	5.96	3.82	12.99	6.12	5.58	3.80	20.84	7.73	6.92
C->18 others	17.45	20.32	14.32	11.06	21.14	13.79	17.48	24.31	28.45	15.30	21.90	20.36	21.09	25.25	12.99	9.23
Total C-18	36.64	42.24	39.98	68.53	57.48	59.23*	48.11	48.93*	44.80	47.38*	40.66	52.68	42.94	52.82	42.35	39.95*
Conversion efficacy of !8:3	13.15	5.82	6.63	32.03	13.97	26.24	18.75	12.18	8.50	27.42	15.05	10.67	8.850	39.45	18.25	17.30*

* The changes are not statistically significant when the same was compared with the isolates grown on nutrient broth media.

identification and taxonomic position. On the basis of morphological, cultural and biochemical characteristics, some of the cultures (isolates no. 1A, 1B, 2B and 6A) belong to genus *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Bacillus* respectively. It was significant to note that the organisms identified are not typically marine oriented. It is also important to note here that the samples were collected along the coastal area, wherein terrestrial runoff is known to intermingle with the marine waters. These organisms were in contact with particulate matter settled onto sediment and grew and multiplied when pockets of optimum parameters are developed. It is proposed that these organisms as such are terrestrial organisms, which are surviving in the sediments. These isolates are also not fastidious and can survive and grow in absence of seawater.

The present study has explored the potential of marine bacterial isolates in accumulating essential fatty acid viz. alpha linolenic acid. Such bacteria can be used as supplement to enrich the animal feed with alpha linolenic acid, which is very essential for the animal to be in a well being state. Further work on the effect of feed supplemented with these bacteria on the animal in terms of lipid profiles and weight gain are being undertaken.

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