APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1986, p. 730–737
0099-2240/86/040730-08$02.00/0
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Vol. 51, No. 4

Biochemical Function and Ecological Significance of Novel Bacterial Lipids in Deep-Sea Procaryotes

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Received 9 September 1985/Accepted 24 January 1986

The fatty acid composition of the membrane lipids in 11 deep-sea bacterial isolates was determined. The fatty acids observed were typical of marine vibrios except for the presence of large amounts of long-chain polyunsaturated fatty acids (PUFAs). These long-chain PUFAs were previously thought to be absent in procaryotes, with the notable exception of a single marine *Flexibacter* sp. In three barophilic strains tested at 2°C, there was a general increase in the relative amount of PUFAs as pressure was increased from a low growth pressure towards the optimal growth pressure. In *Vibrio marinus* MP-1, a psychrophilic strain, PUFAs were found to increase as a function of decreasing temperature at constant atmospheric pressure. These results suggest the involvement of PUFAs in the maintenance of optimal membrane fluidity and function over environmentally relevant temperatures and pressures. Furthermore, since these lipids are essential nutrients for higher taxa and are found in large amounts in the lipids of deep-sea vertebrates and invertebrates, an important, specific role for deep-sea bacteria in abyssal food webs is implicated.

The physical environment of the deep sea is characterized by high hydrostatic pressures and low temperatures (38). Recent investigations have demonstrated that rapidly growing, pressure-adapted bacteria can be readily isolated from the water column, sediments, and intestinal tracts and decaying parts of deep-sea invertebrates (9, 11, 19, 43). These bacteria, termed barophiles, grow optimally or preferentially at pressures greater than 1 atm (1.013×10^5 Pa). The optimal growth pressure of these organisms is usually slightly less than the pressure corresponding to their depth of origin (44). To date, the relative contribution of these abyssal and hadal microorganisms to mineralization, symbioses, and secondary production in the deep-sea remains uncertain (32, 37).

The existence of pressure-adapted bacteria raises interesting questions as to the specific types of physiological and biochemical adaptations necessary for optimization of growth and survival in the abyssal environment. Transcription, translation, enzymic catalysis, and membrane structure and function have all been cited as being sensitive to pressure perturbation (20, 25–27, 46). The low temperatures and high hydrostatic pressures found in the deep sea both have lipid-solidifying effects (5, 26). Thus membranes are one obvious starting point for investigations of biochemical adaptations to the deep-sea environment. Adaptations in lipid-based systems to the effects of low temperature have been well documented (4, 18, 28, 30). In bacteria, plants, and poikilothermic animals, greater amounts of unsaturated fatty acids are incorporated into membrane phospholipids as a function of decreasing growth temperature. This is one mechanism which allows for maintenance of optimal membrane fluidity and function at low temperatures. In gram-negative bacteria, this usually entails increases in relative amounts of monounsaturated fatty acids, as long-chain polyunsaturated fatty acids (PUFAs) have hitherto been considered absent in procaryotes (12, 16, 40). We have previously reported increases in monounsaturated fatty acids as a function of increasing growth pressure in a deep-sea bacterium (8). One objective of this study was to determine the fatty acid composition of membrane phospholipids in a collection of deep-sea bacteria to gain insight into putative lipid-based pressure and temperature adaptations.

It is becoming increasingly apparent that indigenous deep-sea bacteria are metabolically active at in situ temperatures and pressures and are probably quite important in deep-sea trophic dynamics (9–11, 32, 44, 45). Although transport of organic material (such as fecal pellets) to the abyss can be relatively rapid, much of it is degraded extensively during transit (7, 37, 38). Fluxes of fatty acids, for example, decrease rapidly with depth, and much of the organic material which reaches the deep sea is highly refractory (7, 38). One important role which has been suggested for marine bacteria in oceanic food webs is the mobilization of this material into forms which are more easily utilized by other organisms (15, 32, 36). Microorganisms, besides supplying bulk carbon and energy to the food chain, can also contribute specific essential nutrients, such as essential amino acids, essential fatty acids, and B-complex vitamins (15, 36). Close association of barophilic bacteria with the intestinal tracts of deep-sea invertebrates and their contribution to the bulk nutrition of these organisms have been reported (9, 11). As yet, however, the importance of deep-sea bacteria in abyssal trophic dynamics is largely undetermined. Knowledge of the biochemical composition of deep-sea bacteria may provide insight into their possibly important role in deep-sea food webs.

MATERIALS AND METHODS

Bacterial strains and media. Marine broth (type 2216; Difco Laboratories, Detroit, Mich.) and silica gel pour tubes were utilized for the enrichment and isolation of barophilic strains as previously described (43, 44). All strains were maintained at 2°C in marine broth or in solid medium containing marine broth supplemented with 4% gelatin at growth pressures corresponding to the pressure at their depth of origin (Table 1). *Vibrio marinus* MP-1 (ATCC 15381) was purchased from the American Type Culture Collection, Rockville, Md. In experiments with minimal medium, a filter-sterilized basal medium of Baumann and Baumann (2) supplemented with 0.2% glucose, 20 mM MOPS (morpholine propane sulfonic acid [pH 7.0]), and 1 mg of each of 20 amino acids per liter

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TABLE 1. Origin of deep-sea isolates

Isolate	Inoculum[a]	Depth of origin(m)	Geographic location
V. marinus	WS	1,200	Oregon coast
SC2	WS	1,957	San Clemente Basin
AT7	WS	2,500	Aleutian Trench
PE36	ATW	3,584	Patton Escarpment
CNPT3	DA	5,800	Central North Pacific
HS11	SS	5,800	Hawaii coast
PT48	ATW	6,163	Philippine Trench
MT100	ATW	6,790	Marianas Trench
MT115	DA	6,790	Marianas Trench
PT99	DA	8,600	Philippine Trench
MT41	DA	10,476	Marianas Trench

[a] Abbreviations: WS, water sample; ATW, amphipod trap water; DA, decaying amphipod; SS, sediment sample.

was employed. Marine broth buffered with 20 mM MOPS (pH 7.0), boiled for 3 min, cooled, and filtered through a 1 μm glass fiber filter before autoclaving was used in all other experiments.

Preparation of FAMEs and lipid extracts. Cells grown under various hydrostatic pressures at 2°C were harvested in mid- to late-exponential phase (0.5×10^8 to 1×10^8 cells per ml) by centrifugation at $7,719 \times g$, washed in artificial seawater, and frozen at −20°C until lipid extraction. Whole cell methanolysates were used throughout the study for fatty acid methyl ester (FAME) preparation and analyses, unless otherwise indicated, by the technique of Moss et al. (31). Cells were suspended in 5 ml of 5% NaOH in 50% methanol in test tubes sealed with Teflon-lined caps and were heated for 30 min at 100°C. These methanolysates were cooled, brought to pH 2 with 6 N HCl, mixed with 5 ml of 10% boron trichloride in anhydrous methanol, and heated for 5 min at 80°C. After cooling, the FAMEs were extracted into 2 10-ml volumes of chloroform-hexane (1:4). The extracts were evaporated under a stream of nitrogen to a volume of 50 μl and stored at −20°C under nitrogen until analysis. Extraction efficiency was checked by treating the aqueous layer in 5% H_2SO_4 for 30 min at 100°C, retreating with BCl_3 to ensure complete esterification, and re-extracting.

Polar and neutral lipids were prepared from batch cultures by the extraction procedure of Bligh and Dyer as modified by Ames (1), followed by column chromatography on silicic acid (6) (Bio-SilA, 100/200 mesh, Bio-Rad Laboratories, Richmond, Calif.). Neutral lipids were eluted with 10 column volumes of chloroform, and polar lipids were next eluted with 10 column volumes of chloroform-methanol (1:1) and 5 volumes of methanol. Extracts were evaporated under nitrogen, brought up in a small volume of chloroform, and stored under nitrogen at −20°C. Isolated membranes were prepared by the method of Osborn and Munson (34) except that inner and outer membranes were not separated. FAMEs from membranes and phospholipids were extracted by the methods described above.

Analysis of FAMEs. For routine quantitation, a 3% OV-1 column (6 ft [183 cm] by 2 mm inner diameter, 80/100-μm mesh, acid-washed dimethylchlorosilane-treated Chromosorb W support) fitted to a gas chromatograph equipped with a flame ionization detector and an integrator (model 5840A; Hewlett-Packard Co., Palo Alto, Calif.) was used. Samples (0.1 to 1.0 μl) dissolved in hexane-chloroform (4:1) were introduced into the column. The injector temperature was 150°C, the flame ionization detector was at 250°C, and the oven was programmed at a starting temperature of 150°C for 3 min, followed by a temperature increase to 230°C at a rate of 4°C/min. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. Typical run times were approximately 25 min. FAMEs were initially identified by comparison of retention times with known standards. Identifications were confirmed by comparison of sample electron impact mass spectra with the electron impact mass spectra of pure standards and by molecular weight determination obtained from chemical ionization mass spectra.

Mass spectrometry. Gas chromatography-mass spectrometry was performed on a Finnigan model 4021 instrument with a 3% Dexsil 300 column (1.8 m by 2 mm inner diameter, 100/120-μm mesh Supelcoport). Helium was used as the carrier gas at a flow rate of 20.0 ml/min. The injector was at 280°C, the flame ionization detector was at 320°C, and the oven was programmed to run at 150°C for 3 min, followed by an increase in temperature to a maximum of 300°C at a rate of 4°C/min. Electron impact was performed at 70 eV. The temperature of the ion source was 250°C, and the electron multiplier was set at 1,600 V. Scans from 40 to 640 atomic mass units (amu) were run with a scan time of 2 s. Chemical ionization mass spectrometry was performed with NH_3 at a pressure of 0.08 torr (11 Pa), with the same electron multiplier voltage.

Effects of temperature and pressure on fatty acid composition. For pressure experiments, 1 liter of marine broth was inoculated with 3 ml of a bacterial suspension, resulting in a starting concentration of 1.5×10^5 to 3.0×10^5 cells per ml. This was then distributed to six polyethylene bags (160 ml each) which were fitted with polyethylene tubes for sterile subsampling and contained marbles to ensure adequate mixing. The medium was kept chilled and on ice for as much of the operation as possible. The bags were then placed in pressure vessels which were pressurized and placed on a rocker in a bath at 2°C. Periodically subsamples were removed, and cell concentration was determined in a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) as previously described (45). Subsampling decompression time did not exceed 2 min. Cells were harvested in mid- to late-exponential phase (approximately 0.5×10^8 to 1.0×10^8 cells per ml), centrifuged, washed, and frozen until lipid extraction and analysis. In temperature experiments, *V. marinus* MP-1 was grown in Erlenmeyer flasks in marine broth at the indicated temperature at atmospheric pressure.

Bacterial synthesis of PUFAs. Bacteria were grown in a modified, fat-free, basal medium of Baumann and Baumann (2). The medium contained 1× artificial seawater, 20 mM MOPS buffer (pH 7.0), 19 mM NH_4Cl, 0.33 mM K_2HPO_4, 0.1 mM $FeSO_4 \cdot 7H_2O$, 0.2% D-glucose, and 1 mg of each of the 20 major L-amino acids per ml. The media was filter sterilized. Experiments to determine the fatty acid composition of organisms grown in minimal, fat-free medium were performed as described for those in complex media. Incorporation of [^{14}C]acetate into PUFAs was demonstrated by growing the organisms in buffered 2216 medium containing 3 μCi of [2-^{14}C]sodium acetate (54 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) per ml. FAMEs from organisms grown on marine broth containing [2-^{14}C]sodium acetate were prepared as previously described and separated and quantitated on a Varian 1200 gas chromatograph equipped with a flame ionization detector and a 10% SP2330 column (6 ft [183 cm] by 1/8 in. [0.32 cm] inner diameter, 100/120-μm mesh Supelcoport). The column was set initially at 200°C, and after elution of saturated and monounsaturated FAMEs, the column temperature was increased to 230°C. Part of the sample eluting from the column was diverted to a Packard

TABLE 2. Fatty acid composition of deep-sea bacteria

Fatty acid type[a]	Fatty acid composition (wt%) of following deep-sea bacterial isolates at 2°C[b]:										
	CNPT3	MT100	HS11	MT115	MT41	AT7	PE36	*V. marinus*	SC2	PT48	PT99
13:0									6.1	7.3	1.6
14:1	13.7	22.8	10.1	11.4	10.1	15.1	10.8	8.2	1.9	1.0	1.9
14:0	4.8	1.6	3.8	1.0	4.2	23.4	20.9	16.4	4.8	9.8	6.2
15:0[c]									9.5	5.6	6.0
16:1	46.8	49.6	50.8	42.6	35.2	21.0	29.3	34.7	32.3	23.9	26.3
16:0	18.7	13.7	19.7	16.3	23.4	10.8	9.1	12.9	14.3	21.8	11.0
18:1	11.0	9.3	3.7	12.7	6.5	7.4	3.6	6.9	14.1	3.4	7.4
18:0	4.9	3.1	1.2	2.7	4.6	2.5	1.4	3.0	4.6	1.6	2.8
20:5									12.3	25.4	36.7
22:6			10.5	13.4	15.5	19.6	24.7	17.9			

[a] Fatty acids comprising less than 1% of the sample are not included.
[b] Growth pressures of the isolates (in pascals[10^5]) were as follows: CNPT3, 517; MT100, MT115, PT48, and PT99, 552; HS11, AT7, and SC2, 206; MT41, 896; PE36, 345; *V. marinus*, 1.01.
[c] Branched chain.

gas proportional counter (model 894) adjusted to a range setting of 5,000 cpm. Results from the flame ionization detector and gas proportional counter were recorded with a Packard dual pen chart recorder. Peaks were identified by comparison of retention times with those of known standards.

Chemicals. FAME standards were purchased from Sigma Chemical Co., St. Louis, Mo. and Supelco, Bellefonte, Pa. All solvents were organic residue analysis grade (J. T. Baker Chemical Co., Phillipsburg, N.J.). Radiochemicals were purchased from Amersham. All other reagents and media were of the highest grade available.

RESULTS

The fatty acid composition of the psychrophilic deep-sea strains examined in this study was typical of marine vibrio species except for the presence of large amounts of PUFAs found in most of the isolates (Table 2 and 3). Extraction efficiency of FAMEs was 95% or greater. Purified, extracted membranes and purified polar lipids extracted from batch cultures gave identical fatty acid compositions as compared with whole cell methanolysates. Only trace levels of neutral lipids were present. In general, there was a preponderance of even-chain saturated and monounsaturated species as well as the unexpected polyenoic fatty acids. In addition, odd- and branched-chain fatty acids were observed in a few strains. Identifications were initially based on comparison of retention times with those of known standards. These were supplemented by molecular weight determinations by chemical ionization mass spectrometry (Fig. 1), and comparison of electron impact mass spectra with known standards (Fig. 2). The chemical ionization spectrum shows prominent mass peaks of the NH_4 adduct (point A) and the M + H (point B), with point B − 1 (343 − 1 = 342) yielding the molecular weight (Fig. 1). Final identification was confirmed on the basis of molecular weight, identical retention times, and matching electron impact spectra as compared with those of known standards.

Since PUFAs have been considered rare or absent in procaryotes, a number of control experiments were performed to confirm the observed bacterial biosynthesis of these polyenoic lipids. Organisms grown in fat-free minimal medium supplemented with amino acids had fatty acid compositions nearly identical to those of organisms grown in complex medium, including the large amounts of PUFAs (Table 4). In addition, [^{14}C]acetate added to the medium was incorporated into the PUFAs (Fig. 3). To ensure that the growth containers (polyethylene bags) did not introduce

TABLE 3. Fatty acid composition as a function of pressure

Fatty acid type	Fatty acid composition (mean wt % ± SE) of[a]:								
	PE36 at the following growth pressure (Pa [10^5])/*n*			MT41 at the following growth pressure (Pa [10^5])/*n*			PT99 at the following growth pressure (Pa [10^5])/*n*		
	1.01/5	345/4	620/6	620/3	896/5	1,103/3	275/5	552/4	827/6
13:0							4.6 ± 0.4	1.6 ± 0.3	3.4 ± 0.5
14:1	11.7 ± 0.6	10.8 ± 0.6	8.9 ± 0.5	12.7 ± 0.4	10.1 ± 0.7	8.3 ± 0.2	1.0 ± 0.1	1.9 ± 1.1	1.3 ± 0.2
14:0	18.8 ± 1.2	20.9 ± 1.1	24.0 ± 1.0	4.2 ± 0.3	4.6 ± 0.5	3.1 ± 0.3	10.2 ± 0.3	6.2 ± 1.0	9.8 ± 0.2
15:0[b]							6.3 ± 0.2	6.0 ± 0.5	5.2 ± 0.3
16:1	33.5 ± 3.1	29.3 ± 0.8	17.6 ± 0.9	34.9 ± 1.5	35.2 ± 0.4	37.1 ± 1.6	23.8 ± 1.0	26.3 ± 2.1	23.5 ± 1.0
16:0	11.2 ± 1.7	9.1 ± 2.0	10.1 ± 0.9	25.8 ± 0.6	23.4 ± 1.3	21.2 ± 1.1	22.2 ± 1.4	11.0 ± 0.6	20.4 ± 1.1
18:1	6.8 ± 1.0	3.6 ± 1.3	6.3 ± 1.4	7.8 ± 1.1	6.5 ± 1.7	9.3 ± 1.4	5.8 ± 1.1	7.4 ± 0.8	5.8 ± 1.2
18:0	2.3 ± 0.5	1.4 ± 0.9	3.2 ± 0.6	7.4 ± 1.5	4.6 ± 0.4	8.7 ± 2.1	2.2 ± 0.5	2.8 ± 0.8	2.7 ± 0.4
20:5							23.9 ± 0.9	36.7 ± 2.4	27.8 ± 1.1
22:6	15.7 ± 2.3	24.7 ± 2.9	29.7 ± 2.2	7.2 ± 1.3	15.5 ± 1.8	12.3 ± 2.6			
Unsaturation index[c]	137 ± 10	192 ± 8.0	211 ± 12	98 ± 7.0	145 ± 10	128 ± 16	150 ± 5.0	219 ± 12	170 ± 5.0

[a] Doubling time (h) was as follows: PE36 at 1.01 × 10^5 Pa, 8; PE36 at 345 × 10^5 Pa, 6; PE36 at 620 × 10^5 Pa, 21; MT41 at 620 × 10^5 Pa, 36; MT41 at 896 × 10^5 Pa, 26; MT41 at 1,103 × 10^5 Pa, 38; PT99 at 275 × 10^5 Pa, 30; PT99 at 552 × 10^5 Pa, 15; PT99 at 827 × 10^5 Pa, 16.
[b] Branched chain.
[c] Calculated as the sum weight percent multiplied by the number of olefinic bonds for each fatty acid.

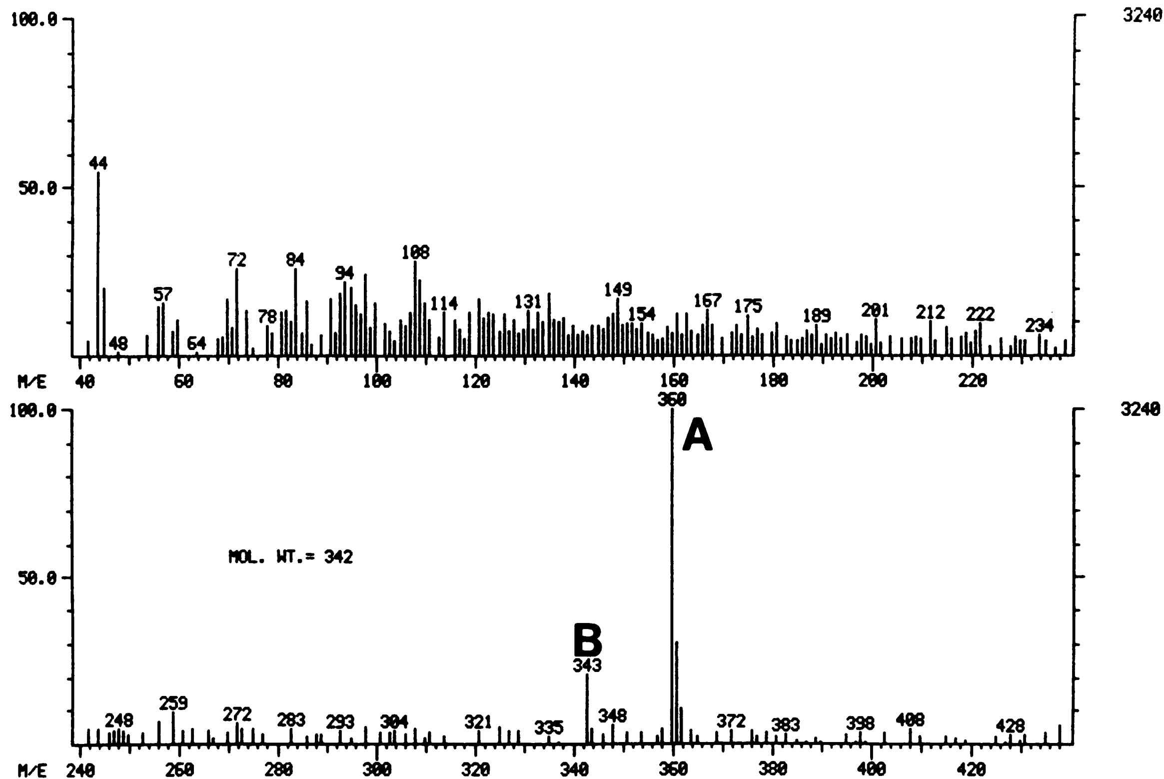

FIG. 1. Chemical ionization spectrum of the C_{22} FAME of MT41. See the text for methods. A, NH_4 adduct; B, M + H. Molecular weight, 342.

artifacts, we grew barophilic organisms in batch culture in Teflon bottles and observed qualitatively identical fatty acid compositions. *V. marinus* had an identical fatty acid composition when grown in either glass flasks or polyethylene bags.

The fatty acid composition of the deep-sea isolates changed as a function of pressure. In general, greater amounts of PUFAs were synthesized at the higher growth pressures. In isolate PE36, an increase in growth pressure was accompanied by increases in the relative proportions of $C_{14:0}$ and $C_{22:6}$. In MT41, greater amounts of $C_{22:6}$ were present at medium and high growth pressures as compared with the low growth pressure. PT99 contained a relatively greater proportion of $C_{20:5}$ at high and medium growth pressures as compared with the low growth pressure. *V. marinus*, in response to decreasing growth temperature, had decreased proportions of $C_{16:0}$ and an increase in the amount of $C_{22:6}$ (Table 5). The effects of decreasing growth temperature on PUFAs in *V. marinus* were analogous to the effects of increasing growth pressure on PUFAs in PE36 (Fig. 4).

DISCUSSION

With the exception of the large amounts of polyenoic fatty acids found in most of the isolates, the fatty acid compositions observed were typical of marine vibrio species. A predominance of $C_{16:1}$ was observed, in concordance with the findings of Oliver and Colwell (33) in a survey of marine vibrios. We also observed large proportions of either $C_{14:1}$ or $C_{14:0}$ in most isolates. This is not surprising, as large amounts of shorter-chain fatty acids in psychrophilic vibrios grown at environmentally relevant temperatures have been observed (18). Four substantially different fatty acid profiles were observed and can be distinguished by the following characteristics: (i) little or no PUFA and ≈50% $C_{16:1}$ (CNPT3, MT100), (ii) $C_{14:1} > C_{14:0}$ and ≈10% $C_{22:6}$ (HS11, MT115, MT41), (iii) $C_{14:0} > C_{14:1}$ and ≈20% $C_{22:6}$ (AT 7, PE 36, *V. marinus* at 2°C), and (iv) some odd- and branched-chain FAMEs and $C_{20:5}$ (SC2, PT48, PT99). The fatty acids were specifically associated with the membrane-bound polar lipids, as would be expected for gram-negative eubacteria.

The presence of long-chain PUFAs in deep-sea bacteria is quite extraordinary. Most of the previous literature indicates that bacteria do not contain PUFAs with more than two double bonds (12, 16, 40). Diunsaturated fatty acids have been found in significant amounts in a number of procaryotes. For instance, marine vibrios have been reported to contain significant quantities of $C_{18:2}$ (33), although it was not clear whether these were of bacterial origin or were absorbed or incorporated from the medium. The only other bacterial group known to contain PUFAs is the cyanobacteria, which contains $C_{18:3}$ (12). One other study has shown unambiguously the presence of a long-chain PUFA in a procaryote. $C_{20:5}$ was found to be a major membrane constituent in a marine *Flexibacter* sp., and its presence was thought to confer greater flexibility to the cell wall of this organism, facilitating its gliding motility (21). Most other studies seem to agree with the general rule that "bacteria do not produce the types of PUFAs characteristic of plants and animals" (16). One salient observation of this investigation is that most of the deep-sea isolates contained large amounts of PUFAs. This may very well be a predominant characteristic of deep-sea heterotrophic eubacteria.

The prevalence of these PUFAs in deep-sea procaryotes raises some interesting questions. For example, do the PUFA biosynthetic enzymes in these bacteria have the same evolutionary origin as those in cyanobacteria or eucaryotes?

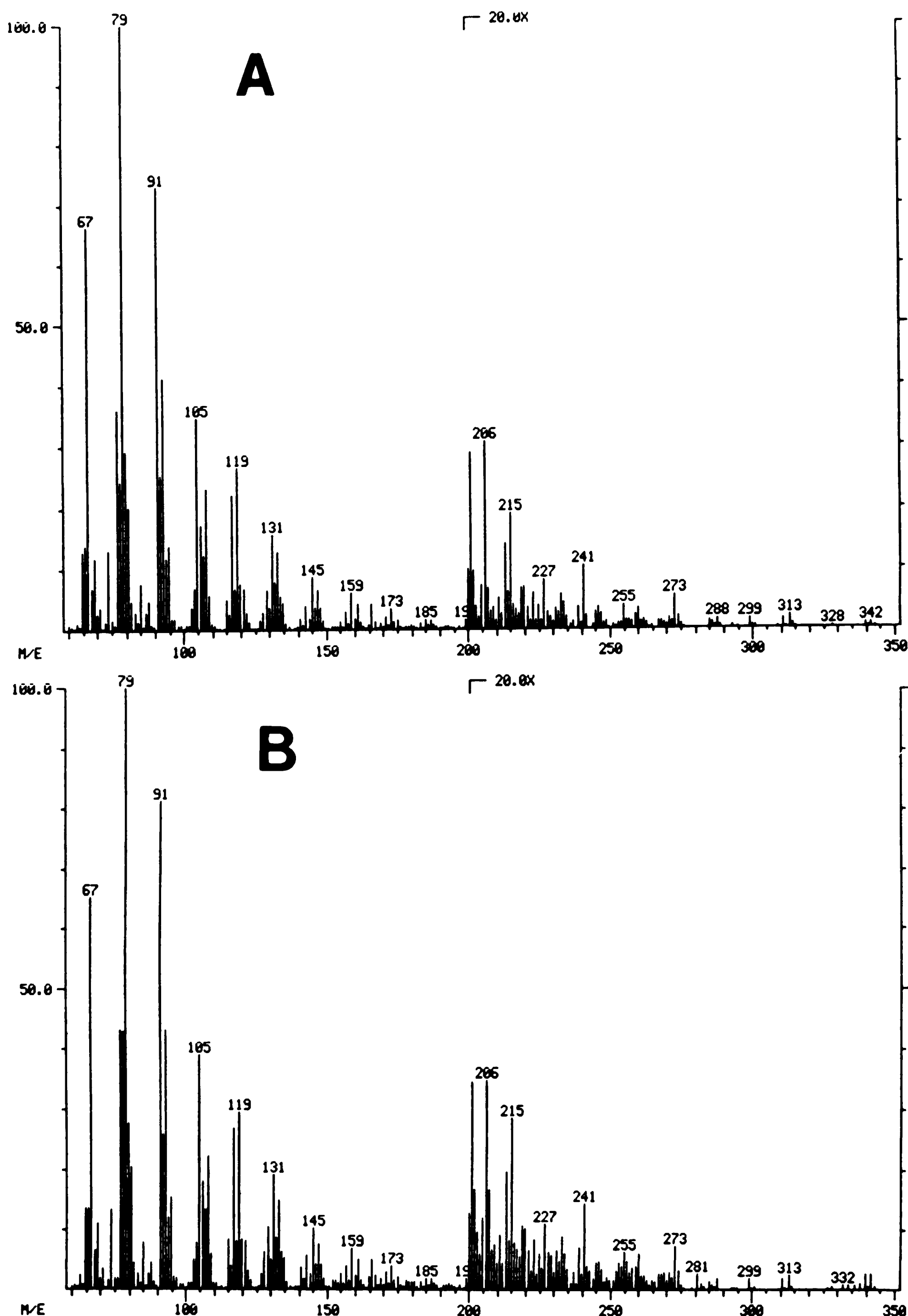

FIG. 2. Electron impact mass spectra of the C_{22} FAME of MT41 (A) and the authentic $C_{22:6}$ FAME (B).

Alternatively, has adaptation to the cold, high-pressure, deep-sea environment resulted in convergent evolution between deep-sea bacteria and photosynthetic organisms with respect to de novo PUFA biosynthesis? It is instructive to note here that the PUFAs we have observed are the types which are characteristically found esterified to the phospholipids of animals and some plants. Although cyanobacteria contain $C_{18:3}$ PUFAs, C_{20} and C_{22} long-chain PUFAs are not found in this group. These facts tend to support the hypothesis that the genes for PUFA biosynthesis evolved separately in deep-sea bacteria. The apparent novelty (for procaryotes) of these PUFAs could make them useful as aids in determining phylogenetic relationships among deep-sea and psychrophilic marine bacteria.

Some recent studies of microbial community structure have relied on specific biochemical indicators, such as fatty acids, to identify various components of the biota (42). This approach is useful in that the community structure is not altered in the analysis, as it could be with methods that require culturing, and therefore can select for specific physiological types. Various participants in microbial consortia can be identified by these techniques. Muramic acid, lipo-

TABLE 4. Fatty acid composition of deep-sea bacteria grown in basal medium[a]

Fatty acid type	Fatty acid composition (mean wt % ± SE) of		
	V. marinus at 1.01×10^5 Pa	PE36 at 345×10^5 Pa	SC2 at 193×10^5 Pa
13:0			4.0 ± 0.2
14:1	3.0 ± 0.2	4.2 ± 0.2	1.2 ± 0.1
14:0	14.9 ± 0.4	14.4 ± 0.8	3.6 ± 0.4
15:0[c]			4.9 ± 0.1
16:1	32.0 ± 1.9	19.3 ± 2.4	17.3 ± 1.2
16:0	16.1 ± 0.5	18.7 ± 3.3	30.4 ± 4.5
18:1	6.5 ± 1.0	10.8 ± 1.7	12.5 ± 2.0
18:0	7.0 ± 0.7	9.2 ± 0.7	11.7 ± 1.2
20:5			14.3 ± 5.4
22:6	20.4 ± 1.0	23.4 ± 4.5	

[a] All experiments were performed at 2°C.
[b] The values represent the mean of three separate samples ± the standard error.
[c] Branched chain.

polysaccharides, and teichoic acids are examples of compounds which are specific to bacteria or bacterial groups and thus are useful as biochemical indicators. The virtual absence of long-chain PUFAs in procaryotic organisms has justifiably led to the use of these lipids as specific indicators of eucaryotic microflora and microfauna (3, 14, 42). In future analyses, however, caution should be used when employing long-chain PUFAs as indicators of microbial community structure in cold or deep waters or sediments, in light of our new data. The possible procaryotic origin of these lipids in cold and deep-sea environments must now be considered.

For metazoans, PUFAs are essential dietary components, and it has been thought that they are synthesized de novo only in photosynthetic organisms (39). Animals must be supplied at least with dienoic or trienoic fatty acids that can then be elongated to the C_{20} and C_{22} PUFAs, which are important structural components of their phospholipids (22, 39). In some marine teleosts, C_{20} and C_{22} PUFAs seem to be essential dietary components, as nutritional studies have shown that activities of $C_{18:3}$ elongation in these organisms are low (22, 39). In terms of deep-sea ecology, this is an important consideration, as both deep-sea vertebrates and invertebrates have been reported to contain large amounts of PUFAs (23, 35). For example, a deep-sea holothurian captured at 4,400 m contained both $C_{20:5}$ and $C_{22:6}$, amounting to 23% by weight of the total fatty acids (23). Degradation of sedimenting organic matter during transit is a limiting factor with repect to food supply to the abyss, and PUFAs are rapidly degraded during transit from the surface to the deep sea (7, 41). There is as much as a 50% loss of PUFAs in the first 500 m of transit (7). Hence it seems unlikely that all the PUFAs of deep-sea metazoans originate from the photic zone. We suggest a specific role for in situ secondary production of essential nutrients (PUFAs) by barophilic bacteria. Other essential nutrients, such as essential amino acids or B-complex vitamins could also conceivably be supplied by these bacteria. Indeed, close association of barophilic bacteria with the intestinal tracts of abyssal holothurians has been observed by Deming et al. (9, 11), and it was estimated that these bacteria contributed about 3% to the total carbon consumption of these invertebrates.

One characteristic common to the bacteria we are studying is the ability to grow well at the low temperatures and high hydrostatic pressures which are found in the deep sea. Since most of the isolates also share the unusual characteristic (for bacteria) of possessing large amounts of PUFAs, it seems reasonable to hypothesize that these lipids are at least partially involved in facilitating growth at these environmental extremes. Previous studies have demonstrated that the lipids of deep-sea fish and Antarctic fish are enriched with C_{20} and C_{22} PUFAs, when compared with those of tropical shallow water origin (35). We note a similar trend, specifi-

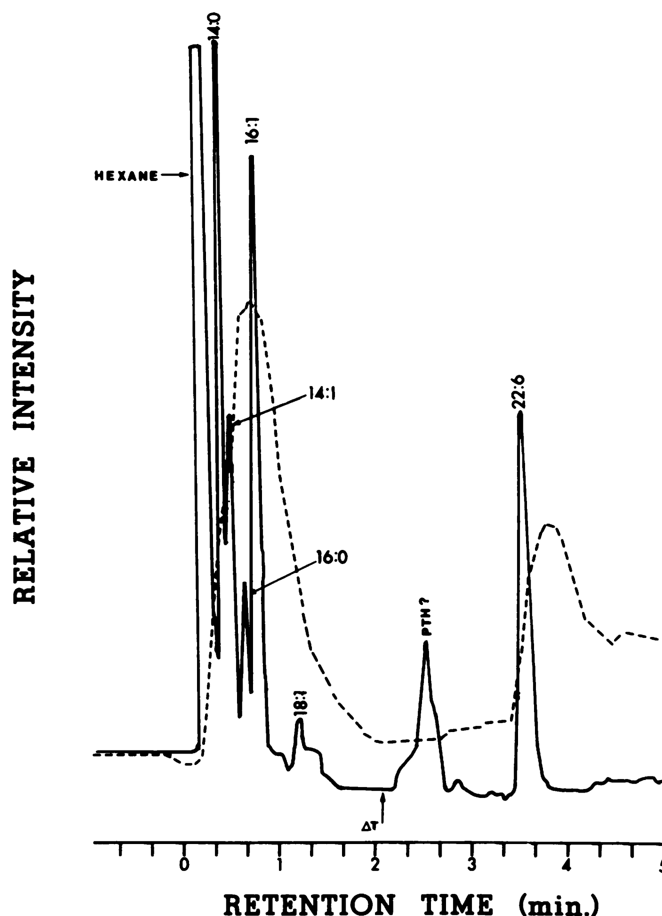

FIG. 3. Radio-gas chromatogram of the FAMEs of PE36 grown on marine broth supplemented with 3 μCi of [^{14}C]sodium acetate per ml. The solid line represents the flame ionization detector response, and the broken line represents the radioactivity of the peaks as measured by a gas proportional counter. PTH, Pthalates. ΔT, Time of temperature increase. See the text for methods.

TABLE 5. Fatty acid composition of *V. marinus* as a function of temperature[a]

Fatty acid type	Fatty acid composition (mean wt % ± SE) of *V. marinus* at (°C)/*n*:		
	2/5	15/4	20/5
14:1	8.2 ± 0.3	4.2 ± 0.2	6.2 ± 0.6
14:0	16.4 ± 0.6	13.9 ± 0.7	15.3 ± 1.3
16:1	34.7 ± 0.6	42.4 ± 0.9	38.0 ± 2.9
16:0	12.9 ± 0.7	18.3 ± 0.8	21.8 ± 1.2
18:1	6.9 ± 0.4	7.6 ± 1.0	8.9 ± 1.8
18:0	3.0 ± 0.8	4.9 ± 0.4	6.5 ± 1.9
22:6	17.9 ± 0.2	8.6 ± 0.6	3.2 ± 0.3
Unsaturation index[b]	157 ± 1.0	105 ± 3.0	72 ± 2.0

[a] All experiments were performed at 10^5 Pa.
[b] The unsaturation index was calculated as described in footnote *c* to Table 3.

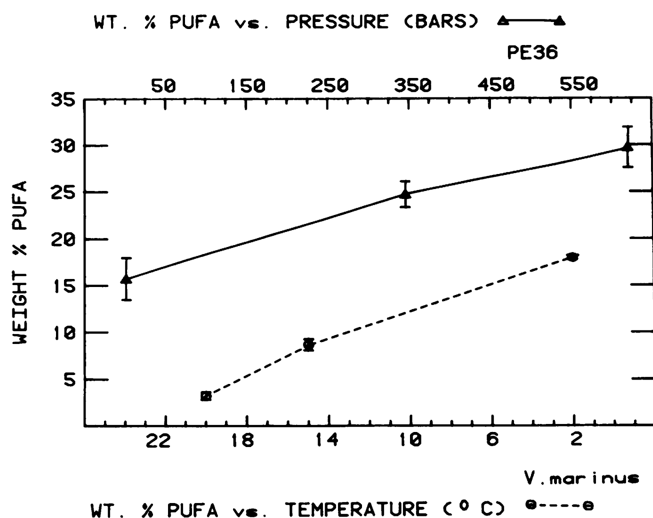

FIG. 4. Weight percent of PUFAs as a function of temperature in *V. marinus* (-----) and of pressure in PE36 (——). One bar equals 1.013×10^5 Pa.

cally, the presence of large amounts of PUFAs in psychrophilic deep-sea bacteria and their virtual absence in mesophilic microorganisms. We have previously reported adaptation in the membrane lipids of isolate CNPT3, which increases the relative proportion of $C_{16:1}$ in response to increasing growth pressure (8). The general increase in PUFAs as the optimal growth pressure is approached appears to be analogous to this response. This trend is also reflected in the increase in the unsaturation index as the optimal growth pressure is approached (Table 3). It should be noted that in two cases (MT41 and PT99) there was a decrease in the relative amount of PUFA at the highest growth pressure as compared with the medium pressure. In these cases, the PUFA maxima corresponded roughly to the optimal growth pressure. In all strains observed, the relative amount of PUFA was higher at both medium and high pressures, when compared with the low growth pressures, as was the unsaturation index.

The presence of PUFAs in *V. marinus* MP-1 most likely represents an adaptation to low temperature rather than to high pressure, as this organism was isolated at a relatively shallow depth and has been reported to be slightly, if at all, barophilic (29). In a previous survey of marine bacterial lipids (33), long-chain PUFAs in *V. marinus* were not detected. However, a different strain of *V. marinus* was studied (PS-207), and this strain is not strictly psychrophilic, as is *V. marinus* MP-1 (17). Increases in PUFAs as a function of decreasing growth temperature have been reported for planktonic crustaceans (13) and the mysid *Neomysis integer* (30), and we have observed a similar response in *V. marinus* MP-1. It could be that membranes adapted to a cold oceanic environment are at least partially pre-adapted to the high hydrostatic pressures found in the deep sea. Perhaps polar submergence (24) into the deep sea occurs in psychrophilic microorganisms associated with higher taxa, which invade the depths from cold, shallow polar regions.

ACKNOWLEDGMENTS

This work was supported by grant OCE 84-16207 from the National Science Foundation and by a contract from Sandia National Laboratories, a U.S. Department of Energy prime contractor.

We thank Larry Sweetman, Stuart Brody, and Gary Coté for assistance with the mass spectroscopy and radio-gas chromatography; K. L. Smith, J. Nevenzel, and G.N. Somero for valuable advice and comments; and Joe Pawlik for aid in PUFA identification and enthusiastic discussion.

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