Extractable Lipids of Gram-Negative Marine Bacteria: Fatty-Acid Composition

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Fatty-acid compositions were determined for 20 strains of marine and estuarine bacteria and two strains representative of terrestrial species. Results showed that the fatty acids of marine bacteria differed little from those of nonmarine organisms, and a primary role for hexadecenoic acid was indicated. Of the 20 strains examined, with the exception of one, the major fatty-acid species were C16, C16:1, and C18:1. Significant differences were observed among the fatty-acid patterns of the various bacterial genera included in the set of 20 strains examined, and rapid differentiation of most of the genera could thus be accomplished. A recently isolated marine species demonstrated a unique fatty-acid pattern wherein branched acids formed the major fatty-acid class. Effects of culture age, growth temperature, and salt concentration of the medium on the fatty-acid profiles were also investigated.

The biochemistry of marine bacteria has only recently been investigated in any detail. Most of the reports on marine bacteria have been concentrated on the distribution, taxonomy, and cytology of these microorganisms. Other than a few reports on the fatty acids and phospholipids of marine bacteria (3, 13, 20, 45; R. Hardy, G. Hobbs, P. R. Mackie, and R. W. Horsley, *Proc. Soc. Gen. Microbiol.* 5:iii), most of which involved only a cursory examination of these compounds, very little information is available on the lipids of marine bacteria. Recently, the phospholipids of a variety of marine and estuarine bacteria were reported by Oliver and Colwell (44); this represented an attempt to compare, for the first time, the lipids of marine organisms with those of terrestrial bacteria. The phospholipids phosphatidylethanolamine, phosphatidylglycerol, and lyso-phosphatidylethanolamine were found to be predominant in all of the organisms examined. The fatty acids of 20 marine and estuarine bacteria were examined in an extension of the phospholipid study.

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MATERIALS AND METHODS

Bacterial strains. Twenty-two bacterial strains were employed (Table 1), 16 of which were of marine origin. Strain labels with the prefix "3S" indicate that the strains were isolated from a sediment sample collected in the Atlantic Ocean. Classification of the sediment isolates was discussed previously (44).

Growth of cultures. All strains except two were grown in an artificial seawater medium (44). The two terrestrial strains, *Vibrio cholerae* ATCC 14033 and *Pseudomonas aeruginosa* ATCC 14216, were grown in a medium identical to that used for the marine isolates but with a 0.5% NaCl solution, rather than artificial seawater, used as diluent. Cells were grown at 25°C to the late logarithmic phase as described previously (44).

Fatty-acid methyl ester preparation. Methods for extraction of lipids from pellets of the harvested cells and freeing the extracts from nonlipid contaminants have been described (44). Fatty-acid methyl esters were prepared from the purified lipid extracts according to the method of Brockerhoff (10). To a sample of the lipid extract dissolved in chloroform was added 1.0 ml of 0.5 N methanolic KOH. After shaking, the mixture was allowed to remain at room temperature for 10 min, after which 1.0 ml of 1.0 N HCl was added. The mixture was again shaken, and the methyl esters were extracted three times with 1.0-ml samples of petroleum ether. The extracts were combined and evaporated under nitrogen, then taken up in carbon disulfide for analysis by gas-liquid chromatography. Fatty-acid methyl esters were kept in glass tubes fitted with Teflon-lined stoppers, under nitrogen, at -10°C.

Fatty acids were hydrogenated in petroleum ether, with palladium chloride as a catalyst. Hydrogenated fatty acids were brominated according to the method of Brian and Gardner (9) to test for the presence of

TABLE 1. *Strains used in the study*

Strains ^a	Habitat ^b	Source
<i>Vibrio alginus</i> ATCC 14390	M	Associated with marine kelp. ZoBell and Upham (56).
<i>V. marinofulvus</i> ATCC 14395	M	Seawater. ZoBell and Upham (56).
<i>V. alginolyticus</i> 166-70	E	Received from R. Sakazaki (National Institute of Health, Tokyo) as strain 166-70.
<i>V. parahaemolyticus</i> ATCC 17802	E	
<i>V. marinus</i> strain PS-207	E	Skin of a Pacific cod. Colwell (16).
<i>V. cholerae</i> ATCC 14033	T	El Tor Station, 1930. Suggested neotype.
<i>Agrobacterium stellulatum</i> ATCC 15215	M	Knösel.
<i>Achromobacter aquamarinus</i> ATCC 14400	M	Seawater. ZoBell and Upham (56).
<i>Spirillum linum</i> ATCC 11336	M	Seawater. Rittenberg.
<i>Pseudomonas perfectomarinus</i> ATCC 14405	M	Seawater and marine mud. ZoBell and Upham (56).
<i>Pseudomonas aeruginosa</i> ATCC 14216	T	
<i>Photobacterium fischeri</i> ATCC 7744	M	
<i>Arthrobacter marinus</i> ATCC 25374	M	Seawater. Received from G. Jones.
10RR10	E	Water sample from Rhode River, Md. by T. Kaneko.
3S1	M	Marine sediments.
3S2	M	Marine sediments.
3SE5	M	Marine sediments.
3SE7	M	Marine sediments.
3SE8	M	Marine sediments.
3SC1	M	Marine sediments.
3ST2	M	Marine sediments.
3ST3	M	Marine sediments.

^a ATCC, American Type Culture Collection, Rockville, Md.^b M, Marine; E, estuarine; and T, terrestrial.

cyclopropane fatty acids. Silyl ether derivitization of the methyl esters was performed with Sil-Prep, obtained from Applied Science, Inc. (State College, Pa.), for detection of hydroxy fatty acids.

Gas-liquid chromatography. Fatty-acid methyl esters were examined by using a dual-column gas chromatography apparatus (model 7461, Packard Instrument Co.) equipped with dual flame ionization detectors. Three stainless steel columns (1/8 in by 6 ft; approximately 0.3 by 180 cm), obtained from Applied Science, Inc., were routinely used for examination of the fatty-acid methyl esters. Ethylene-glycol adipate (10% EGA) and diethyleneglycol succinate (10% DEGS), both on 100- to 120-mesh Gas-Chrom Q, were used as polar columns, and 3% methyl silicone (SE-30), on 80- to 100-mesh Gas-Chrom Q, was used as a nonpolar column. The carrier gas used was ultra-high-purity nitrogen at a flow rate of 50 ml/min. Column operating temperatures were 180 C for the DEGS and SE-30 columns and 190 C for the EGA. Injection port and detector temperatures were 15 C above that of the column in use.

The following fatty-acid methyl ester standards were used for comparison: straight-chain saturated C8, C10, C12, C14, C15, C16, C18, C20, C22, and C24; straight-chain unsaturated C14:1, C16:1, C18:1, C18:2, C18:3, and C22:1; *iso*-branched iC14, iC16, iC18, and iC20; *anteiso*-branched aC15, aC17, aC19, and aC21; hydroxy-substituted OH-C14, OH-C16, and OH-C18; and cyclopropane cycC19.

Quantitation of the fatty acids was obtained by

using a Hewlett-Packard model 3373B electronic integrator. Peak areas provided by the integrator were calculated from the total area, and from this was calculated the percentage each peak represented.

Identification of fatty acids. Identification of the fatty acids found in the lipid extracts was based on retention times on the three columns, relative to methyl myristate, by comparison of the relative retention values to equivalent chain-length graphs (1, 2), by hydrogenation of the methyl esters to convert unsaturated species to the saturated analogs, by preparation of bromine derivatives of the hydrogenated acids to remove cyclopropane fatty acids, and by co-chromatography with the 28 fatty-acid methyl ester standards.

Effect of growth temperature on fatty-acid composition. A study of the effect of temperature on the fatty-acid profile of *Vibrio marinus* strain PS-207 was made. The organism was grown at 25 and 15 C, ± 0.5 C. When the cells reached late logarithmic phase, they were harvested. The lipids were extracted and purified, and the fatty-acid methyl esters were prepared and examined by gas-liquid chromatography.

Effect of culture age on fatty-acid composition. The marine isolate 3SE8 was grown for 3.5-, 5-, 6.5-, 9-, and 96-h time intervals, corresponding to points in the logarithmic, early stationary, and late stationary phases of growth, at 25 C. After cell harvest and lipid extraction, the fatty acids were prepared and examined.

The marine isolates 3S2 and 3SE7 were similarly

examined for effect of age on fatty-acid composition, each strain being grown for periods corresponding to logarithmic and middle stationary growth phases.

Effect of salt concentration on fatty-acid composition. To investigate the effects of salt concentration on the fatty-acid composition of the marine micro-organisms, we prepared five 250-ml side-arm flasks, each with 75 ml of medium containing 0.1% proteose peptone (Difco) and 0.1% yeast extract (Difco), but with varying concentrations of "3-salts" solution, according to the following scheme. Flask one contained 0.4 M NaCl, 0.01 M KCl, and 0.028 M $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, and was designated complete; flask two contained 0.2 M NaCl, 0.005 M KCl, and 0.014 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and was designated one-half strength; flask three contained 0.1 M NaCl, 0.0025 M KCl, and 0.007 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and was designated one-quarter strength; flask four contained 0.05 M NaCl, 0.00125 M KCl, and 0.0035 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and was designated one-eighth strength; and flask five contained distilled water. All media were adjusted to pH 7.3 and were inoculated by picking a colony from a streak plate of the marine isolate 3SE8 to the flask. The cultures were incubated at 27 C on a reciprocal shaker. At periodic intervals, samples were removed to monitor growth by reading optical density at 660 nm.

After harvesting the cells, we extracted and purified the pellets by following standard methods and prepared the fatty-acid methyl esters.

Chemicals. All extractions and derivitizations were performed by using glass-distilled chloroform, methanol, and petroleum ether obtained from Bodman Chemicals (Narberth, Pa.). Fatty-acid methyl ester standards were obtained from Supelco, Inc. (Bellefonte, Pa.) and Applied Science Laboratories (State College, Pa.). All other reagents and medium components were of the highest grade available.

RESULTS

In Table 2 are listed the results of the fatty-acid composition analyses of the 22 strains examined. Most striking was the predominance of the C16:1 component, the major fatty acid in extracts of 14 of the 22 strains. Of the remaining eight strains, C18:1 was the major fatty acid found in seven strains, with only the marine isolate 3SE5 presenting a significantly different fatty-acid pattern. With the exception of strain 3SE5, all organisms revealed C16, C16:1, and C18:1 to be the major fatty acids. A composite of the fatty-acid profiles is provided in Fig. 1-3. As would be expected for fatty-acid profiles of true bacteria, the saturated acids were predominantly of even carbon number. Acids of short chain length occurred only in very small amounts, and no acids of greater than 20 carbons were present (43). All of the 22 strains examined possessed the straight-chain C12, C13, C14, C15, C16,

C17, C18, and the unsaturated C16:1 and C18:1 fatty acids. C17:1 fatty acid was present in all but one strain.

Of the variety of fatty acids detected among the strains tested, only one is not usually found in bacterial lipids. The acid found to occur in 16 of the 22 strains in amounts ranging from traces to over 12% of the total had a carbon number of 19.0 on EGA columns, 19.3 on DEGS, and 18.9 on SE-30. Whereas such values would suggest an identity of normal C19, this component was found to be an unsaturated species. Hydrogenation of samples containing this acid caused a total loss of the peak with a proportional increase in the C18 component. The possibility of its being a hydroxy fatty acid was ruled out, as these are not lost on hydrogenation. The trimethylsilyl ether reagents failed to affect relative mobility of the fatty acid. Similarly, the possibility of its being a cyclic acid was negated, as only relatively vigorous hydrogenation, by using platinum oxide in the presence of glacial acetic acid, would result in the opening of the cyclopropane ring (14). Relative retention of this component did not correspond to the cyclic acids.

Based on retention time on several columns, conversion to C18 on hydrogenation, and identical mobility with C18:2, the unknown component was identified as a straight-chain C18:2. Jamieson (29) reported an equivalent chain length on EGA of 18.98 for 18:2 ω 6, which corresponds well with the observations of this study for the acid in question, although the positions of the double bonds were not determined.

From examination of Table 2 and Fig. 1-3, it can be seen that the C16, C16:1, and C18:1 acids formed the major portion of the total fatty-acid composition of the *Vibrio* strains, with C16:1 predominating in all strains except the estuarine isolate, strain 10RR10, in which a slightly higher amount of C18:1 occurred. Among the vibrios tested, C16:1 ranged from 35 to 50%, C16 from 11 to 31%, and C18:1 from 9 to 38%, with averages of 41, 20, and 25.4%, respectively. The quantitative variations in composition were reflected in the ratios of C16:1 + C18:1/C16 + C18, which ranged from 1.7 to 6.4. Significant quantities of C18:2 were found to occur in *V. marinus* strain PS-207, and lesser amounts occurred in *V. alginus* ATCC 14390 and *V. marinofulvus* ATCC 14395. With the exception of C12, only a very small proportion of the total fatty-acid composition existed as acids of less than C14 in any of the strains, although a number of saturated, unsat-

urated, and branched short-chain acids were found to be present.

Pseudomonas perfectomarinus ATCC 14405 and *P. aeruginosa* ATCC 14216 were found to possess surprisingly similar fatty-acid composition. Extracts of both strains contained C18:1 as the major acid, with C16:1 and C16 being the second and third most prominent. Only trace amounts of any fatty acid of less than 14 carbons were found, with >95% of the fatty acid composition being the three major acids. The C18:1 acid did occur in greater amounts in the strain of *P. aeruginosa*, as did those acids of less than 16 carbons.

A significant difference in the fatty-acid profile of *Arthrobacter marinus* ATCC 25374, when compared with the other strains examined, was the presence of cyclopropane C17. Bromination confirmed the presence of cyclic C17, but cyclopropane C19 was not detected. Other than the occurrence of cyclic C17, the fatty acids of *A. marinus* ATCC 25374 were similar to those present in the *Vibrio* strains examined, as was the C16:1 + C18:1/C16 + C18 ratio.

The fatty-acid composition of *Agrobacterium stellulatum* ATCC 15215 showed a significantly high percentage of C18:1. This fatty acid alone accounted for >93% of the total fatty acids. Little C16 or C16:1 was detected, although these fatty acids were the two next most abundant species, accounting for 4.1 and 1.2%, respectively, of the total fatty acids. Of the strains examined, only *A. stellulatum* ATCC 15215 did not contain detectable amounts of C17:1. Because of the high C18:1 content, the unsaturated-to-saturated C16 + C18 ratio was extremely high (18.6), and the percentage of total unsaturated acids exceeded 94%.

Achromobacter aquamarinus ATCC 15215 revealed a fatty-acid pattern with a predominance of C18:1 (65%) and lower, but significant, amounts of C16 and C16:1. The unsaturated-to-saturated C16 + C18 ratio was, in addition, quite high, indicating the rather low concentration of C16.

The fatty-acid profile of *Spirillum linum* ATCC 11336 revealed a pattern similar to that obtained for the vibrios. The C16 and C16:1 contents were in the same range, with the C18:1 percentage only slightly higher than that found among the vibrios. The unsaturated-to-saturated C16 + C18 ratio was higher than that of the marine vibrios but was similar to ratios obtained for the estuarine vibrios.

With few exceptions, the fatty-acid composition of *Photobacterium fischeri* ATCC 7744 was found to be almost identical to that of the

Vibrio strains tested. The amounts of the three major acids, C16:1, C18:1, and C16, were in proportions similar to those of the vibrios, as were the unsaturated-to-saturated C16 + C18 ratios. No acids of carbon number less than 12 were detected, with the exception of one acid which was not detected in any of the other strains examined and was believed to be a mono-unsaturated C9, occurring in significant quantity (5.3%) in *Photobacterium fischeri* ATCC 7744. Identification of the fatty acid was based on relative retention time (0.19 relative to methyl myristate), indicating a carbon number of 9.2, and loss on hydrogenation. The component was not observed to revert to a C9 acid on hydrogenation, however, and the carbon number of C9:1 should be 9.4 on EGA. Lower relative retention time could result from branched C9:1, but hydrogenation would still cause a reversion of this component to a branched C9, not seen in this instance. Another possibility to be considered is that the component may not be a fatty acid but an aldehyde or fatty aldehyde (4, 23). Aldehydes have been shown to be a requirement for the luminescent system in bacteria, although the role played by these compounds is not fully understood (26). Such compounds have been shown by Gray (22) to have retention times slightly less than the corresponding fatty-acid methyl ester, as is the case for the dimethyl-acetal derivatives of aldehydes, formed by the procedures commonly used for the methylation of fatty acids. Unsaturated, as well as branched, aldehydes are also known, and the possibility that the unknown "C9" component exists as one of these aldehydes must be considered.

Among the strains recently isolated from marine sediment, a diversity of quantitative patterns was observed, although qualitatively the fatty-acid profiles were similar (see Fig. 1-3). Except for isolate 3SE5, the major fatty acids detected were C16:1 (30-52%), C16 (15-28%), and C18:1 (14-31%). All of the isolates, except 3SE5 and 3S2, were identified as *Vibrio* spp. (44), and the ranges given were similar to those obtained for the reference *Vibrio* strains examined in this study, as were the average values of the three acids (C16:1 = 39%, C16 = 20.5%, C18:1 = 22%). The unsaturated-to-saturated C16 + C18 ratios among the marine isolates (3SE5 excluded) were remarkably similar, ranging from 2.2 to 4, as were the percentages of total unsaturated acids.

Although fatty-acid compositions of the majority of the sediment isolates were similar, a

TABLE 2. Fatty-acid composition of lipids extracted from strains of species of the genus *Vibrio* and other genera of bacteria and from eight marine sediment isolates

Fatty acid	Fatty-acid Composition (% of total)						
	<i>V. alginus</i> ATCC 14390	<i>V. marino- fulvus</i> ATCC 14395	<i>V. marinus</i> PS-207	<i>V. alginol- yticus</i> 166-70	<i>V. parahae- molyticus</i> ATCC 17802	<i>V. cholerae</i> ATCC 14033	IOOR10
10	tr ^a	0.1	0.2	tr		tr	tr
br11		tr	tr				
11	0.1	0.2	0.2	tr			tr
br12			tr				
12	3.5	0.3	5.4	tr	0.1	0.1	tr
br13		tr			tr		
13	tr	0.2	0.5	tr	tr	tr	tr
13:1	tr						
i14		tr		tr	tr	0.1	0.4
14	2.6	1.9	3.7	1.3	1.5	4.2	1.2
14:1	0.2	0.2	1.2	0.4	0.4	0.4	0.2
br15				tr	tr		0.3
15	0.2	2.6	0.7	0.5	1.2	0.2	0.3
15:1	tr	1.0	0.2	0.3	0.5		0.6
i16				0.7	tr	1.1	3.7
16	31.0	30.4	19.2	13.1	11.2	22.5	12.5
16:1	37.5	44.8	39.2	50.0	40.2	41.3	35.6
br17							0.3
17	tr	1.6	tr	0.2	2.0	tr	0.3
17:1	tr	6.5	tr	1.7	3.8	1.1	3.2
i18							0.2
18	3.1	1.1	1.6	0.5	1.0	1.1	3.0
18:1	19.8	9.0	15.4	31.2	38.0	27.9	36.7
18:2	1.9	tr	12.4				2.1
16:1 + 18:1/							
16 + 18	1.7	1.7	2.6	6.0	6.4	2.9	4.7
% Unsaturated	59.2	61.6	68.4	83.6	82.9	70.7	78.4

Fatty acid	Fatty-acid Composition (% of total)						
	<i>P. perfectio- marinus</i> ATCC 14405	<i>P. aerugi- nosa</i> ATCC 14216	<i>A. stellu- latum</i> ATCC 15215	<i>A. aqua- marinus</i> ATCC 14400	<i>S. linum</i> ATCC 11336	<i>P. fischeri</i> ATCC 7744	<i>A. marinus</i> ATCC 25374
10	tr	tr	tr	tr	tr		tr
br11							tr
11	tr	tr	tr	tr	tr		0.1
br12		tr					tr
12	0.1	tr	tr	tr	tr	0.3	0.1
12:1	tr						
br13		tr	tr	tr		tr	
13	tr	tr	tr	0.1	tr	0.6	0.1
13:1		tr					
i14	0.1		tr	tr	tr	tr	tr
14	1.1	1.0	0.1	0.2	0.8	7.9	0.1
14:1	0.1	tr	tr	0.1		0.7	tr
br15					tr		
15	0.7	tr	0.1	0.1	tr	0.2	0.2
15:1			0.1	tr		0.1	tr
i16	0.1	0.1			0.1		
16	22.4	16.9	4.1	11.8	16.9	18.8	26.5
16:1	24.2	34.4	1.2	21.4	40.5	39.4	41.7

Table 2 (continued)

Fatty acid	Fatty-acid Composition (% of total)						
	<i>P. perfectomarinus</i> ATCC 14405	<i>P. aeruginosa</i> ATCC 14216	<i>A. stellulatum</i> ATCC 15215	<i>A. aquamarinus</i> ATCC 14400	<i>S. linum</i> ATCC 11336	<i>P. fischeri</i> ATCC 7744	<i>A. marinus</i> ATCC 25374
br17					tr		
17	0.4	0.3	0.2	0.6	0.4	0.2	0.7
17:1	1.0	0.1		0.2	0.2	0.2	0.2
cyc17							5.1
18	0.5	0.5	1.0	0.2	0.2	tr	0.3
18:1	48.6	46.2	93.2	65.2	40.6	18.0	24.4
18:2	0.9	0.4		0.1	0.1	8.1	0.3
19	tr						
19:1	tr						
Misc						5.3 (C9:1)	
16:1 + 18:1/ 16 + 18	3.2	4.6	18.6	7.3	4.7	3.5	2.5
% Unsaturated	74.8	81.1	94.5	87.0	81.4	72.1	66.6

Fatty acid	Fatty-acid Composition (% of total)							
	3S1	3S2	3SE5	3SE7	3SE8	3SC1	3ST2	3ST3
10		0.1	tr	0.6	tr	tr	1.3	tr
br11								tr
11	tr	0.3	tr		tr	0.1	tr	tr
11:1	tr							
br12					0.1			
12	1.2	0.1	0.2	1.9	3.8	1.7	0.6	0.9
br13		tr	19.9		tr			0.2
13	0.1	tr	1.7	0.2	tr	0.2	2.3	tr
13:1							1.6	
il14	0.6	tr	2.0	0.2	1.6	0.3		tr
14	0.8	0.8	4.2	1.4	1.1	1.0	2.2	1.9
14:1	0.4	0.7		0.8	0.2	0.3	1.0	1.1
br15			17.5					0.2
15	0.3	0.4	6.5	0.7	0.6	0.3	0.6	0.3
15:1		0.3	0.9					
il16	3.4			2.6	6.9	3.0	tr	1.4
16	21.6	27.9	13.8	15.8	19.2	15.4	25.0	18.7
16:1	45.6	52.4	18.6	49.9	48.4	43.7	30.2	39.2
17	0.1	0.1	1.4	1.3	0.1	0.2	0.4	0.9
17:1	0.3	0.8	8.3	0.2	1.2	0.5	1.1	0.3
il18				0.3				0.3
18	2.5	1.2	0.9	1.3	0.5	1.0	2.6	1.0
18:1	23.2	14.7	4.0	17.5	16.0	26.9	31.1	27.2
18:2	tr	0.4		5.6	0.1	5.5		3.9
19								2.3
16:1 + 18:1/ 16 + 18	2.9	2.3	1.5	3.9	3.3	4.0	2.2	3.4
% Unsaturated	69.4	69.2	31.9	74.1	66.0	76.7	65.0	71.7

^a Less than 0.1% of total.

few differences were detected and merit discussion. Isolate 3ST2 exhibited almost equal amounts of C16 and C16:1, with the result that C18:1 was the major acid in the profile

obtained for this strain, the only sediment isolate to demonstrate such a pattern. Isolate 3ST3 was the only sediment isolate to demonstrate the presence of C19 but was otherwise

FIG. 1. Fatty-acid profiles. See text for procedural details. A, *Vibrio alginus* ATCC 14390; B, *Vibrio marinofulvus* ATCC 14395; C, *Vibrio parahaemolyticus* ATCC 17802; D, *Vibrio alginolyticus* 166-70; E, *Vibrio marinus* PS-207 (25 °C); F, strain 10RR10; G, *Vibrio cholerae* ATCC 14033.

similar in fatty-acid profile to the other sediment isolates and the *Vibrio* strains. The presence of C15:1 appeared to be characteristic of the vibrios examined, and isolates 3SE5 and 3S2 also contained this acid. Unlike the other sediment isolates, the two organisms lacked the *iso*-branched C16 acid, occurring in small, but significant, quantities in all but one of the other sediment isolates. Isolate 3S2 was, in general, similar to the other vibrio and marine sediment isolates examined.

The one exception to the general fatty-acid pattern observed among the sediment isolates

was that obtained for isolate 3SE5. Among the acids detected in extracts prepared from this organism were quantities of branched acids, the most abundant fatty-acid group (39.4%). The main fatty-acid component was an *iso*-branched C13, constituting almost 20% of the fatty-acid total, with a branched C15 also occurring in significant quantity (17.5%). The C16 and C16:1 acids amounted to only slightly greater than 32%. Unsaturated acids, never less than 59% for the other 21 strains, totaled 31.9%. Another unusual finding was the large (8.3%)

FIG. 2. Fatty-acid profiles. See text for procedural details. A, *Pseudomonas perfectomarinus* ATCC 14405; B, *Pseudomonas aeruginosa* ATCC 14216; C, *Photobacterium fischeri* ATCC 7744; D, *Spirillum linum* ATCC 11336; E, *Achromobacter aquamarinus* ATCC 14400; F, *Agrobacterium stellulatum* ATCC 15215; G, *Arthrobacter marinus* ATCC 25374.

FIG. 3. Fatty-acid profiles. See text for procedural details. A, Strain 3S1; B, strain 3S2 (4 h); C, strain 3SE7 (4 h); D, strain 3SE8 (5 h); E, strain 3SC1; F, strain 3ST2; G, strain 3ST3; H, strain 3SE5.

quantity of C17:1 detected and low (4.0%) content of C18:1.

Effect of culture age on fatty-acid composition. Extracts of marine sediment isolate 3SE8 were prepared from cells grown for 3.5, 5, 6.5, 9 and 96 h, and the effects of culture age on the fatty-acid composition of the marine bacterium were determined (see Fig. 4). Results obtained after quantitation of the fatty acids are listed in Table 3. With few exceptions, changes in the fatty-acid composition were relatively minor. Of the major acids, only C16:1 was found to increase significantly, with

FIG. 4. Effect of culture age on the fatty-acid profile. See text for procedural details. The strain used was 3SE8. A, 3.5 h; B, 5 h; C, 6.5 h; D, 9.5 h; E, 96 h.

TABLE 3. Effect of culture age on fatty-acid composition of strain 3SE8^a

Fatty acid	Growth of strain 3SE8 after				
	3.5 h	5 h	6.5 h	9 h	96 h
10	tr ^b	0.2	tr	tr	tr
11	1.0	tr	tr	tr	tr
i12	0.3	0.1	tr	tr	—
12	2.1	4.2	4.5	1.8	4.4
13	0.1	0.2	0.2	tr	0.5
i14	6.7	0.9	0.9	tr	0.2
14	0.9	1.4	1.0	1.3	1.8
14:1	0.3	0.3	0.2	0.1	0.3
15	0.7	0.8	0.6	0.4	1.1
i16	13.6	4.5	5.2	0.4	1.7
16	16.3	19.4	20.9	22.2	18.8
16:1	35.4	46.8	43.6	50.6	44.8
17	0.4	0.4	0.6	0.6	0.3
17:1	1.1	1.0	0.6	0.3	0.5
18	1.8	0.6	0.6	0.9	0.9
18:1	18.5	17.2	20.6	21.1	22.0
18:2	0.7	2.0	0.5	1.4	2.7
16:1 + 18:1/ 16 + 18	3.0	3.2	3.0	3.1	3.4
% Unsaturated	56.0	67.2	65.5	73.6	70.3

^a Cells were grown at 25 °C and harvested at indicated times. Results expressed as percent of total fatty-acid composition.

^b Less than 0.1% of total.

C16 increasing slightly; both leveled off after 5 h. In the case of C18, a small decrease was observed after the initial sampling, with a leveling off also at about 5 h. The percentage of C18:1 increased slightly over the entire growth period, but the total change was small. Although *iso*-branched acids were detected only at low levels (except for *iso*C16 in the logarithmic phase), a slight increase in the

parent acid paralleled the decrease of the *iso*-branched acid.

Fatty acids isolated from extracts prepared from marine sediment isolate 3SE7 after 4 and 15.5 h of growth are given in Table 4. Changes in the fatty-acid content over the growth periods studied, i.e., logarithmic and stationary phases of growth, were minor (see Fig. 5). Increases in both C16:1 and C16 were observed in extracts from 3SE7; these increases were approximately equivalent to those observed for 3SE8 over the same time periods. As noted for 3SE8, the C18 composition remained constant, although the C18:1 content of 3SE7 decreased

with age. Unlike isolate 3SE8, all of the branched acids increased with age. Unsaturated C16 did not decrease as growth progressed toward the stationary phase. Also, unlike 3SE8, the percentage of total unsaturated fatty acid decreased.

A similar study of the fatty acids of isolate 3S2 is summarized in Table 5. As noted for isolates 3SE8 and 3SE7, the most interesting aspect of the study of the fatty acids of isolate 3S2 was the lack of change in any of the acids with age (see Fig. 6). Unlike isolates 3SE7 and

TABLE 4. *Effect of culture age on fatty-acid composition of strain 3SE7^a*

Fatty acid	Growth of strain 3SE7 after	
	4 h	15.5 h
10	0.6	0.7
11		tr ^b
12	1.9	2.0
13	0.2	0.3
i14	0.2	0.5
14	1.4	1.9
14:1	0.8	0.5
15	0.7	0.6
i16	2.6	3.8
16	15.8	19.9
16:1	49.9	53.8
17	1.3	1.0
17:1	0.5	tr
i18		0.2
18	1.3	1.2
18:1	17.5	13.5
18:2	5.6	0.3
16:1 + 18:1/ 16 + 18	3.9	3.2
% Unsaturated	74.1	68.2

^a Cells were grown at 25 °C and harvested at indicated times. Results expressed as percent of total fatty-acid composition.

^b Less than 0.1% of total.

FIG. 5. *Effect of culture age on the fatty-acid profile. The strain used was 3SE7. See text for procedural details. A, 4 h; B, 15 h.*

TABLE 5. *Effect of culture age on fatty-acid composition of strain 3S2^a*

Fatty acid	Growth of strain 3S2 after	
	4 h	26 h
10	0.1	0.2
11	0.3	0.3
12	0.1	0.2
br13	tr ^b	tr
13	tr	0.4
i14	tr	
14	0.8	0.6
14:1	0.7	0.2
i15		0.5
15	0.4	1.5
15:1	0.3	0.6
i16		0.3
16	27.9	24.2
16:1	52.4	51.9
17	0.1	1.3
17:1	0.8	4.6
18	1.2	1.0
18:1	14.7	12.2
18:2	0.4	tr
16:1 + 18:1/ 16 + 18	2.3	2.6
% Unsaturated	69.2	69.5

^a Cells were grown at 25 °C and harvested at indicated times. Results expressed as percent of total fatty-acid composition.

^b Less than 0.1% of total.

FIG. 6. *Effect of culture age on the fatty-acid profile. Strain used was 3S2. See text for procedural details. A, 4 h; B, 26 h.*

3SE8, however, slight decreases were found in C16 and C18 as well as in C16:1 and C18:1. The small changes observed were reflected by both the C16 + C18 unsaturated-to-saturated ratio and the percentage of total unsaturated fatty acid, neither of which showed significant change.

Temperature effect on fatty-acid composition. The effect of fatty-acid composition of lowered growth temperature for *Vibrio marinus* strain PS-207, i.e., a drop in temperature from 25 to 15 C, is summarized in Table 6 (also see Fig. 7). The main difference observed was an appreciable increase in percentage of C16:1. Of interest, however, was a decrease observed in C18:1 content, indicating selective unsaturated-acid increase. With the exception of C17, all saturated acids decreased with the lowered growth temperature, although a uniform increase in unsaturated species was not observed. Both the C16:1 + C18:1/C16 + C18 ratio and the percentage of total unsaturated acid figures indicated a trend of increased unsaturated and decreased saturated fatty acids with lowered growth temperature.

Effect of salt concentration on fatty-acid

TABLE 6. Effect of temperature on the fatty-acid composition of *V. marinus* PS-207^a

Fatty acid	Total fatty acid growth (%) at	
	25 C	15 C
10	0.2	tr ^b
br11	tr	tr
11	0.2	tr
br12	tr	
12	5.4	4.1
br13		tr
13	0.5	tr
14	3.7	3.4
14:1	1.2	1.8
15	0.7	0.2
15:1	0.2	tr
16	19.2	16.9
16:1	39.2	57.5
17	tr	3.0
17:1	tr	0.3
18	1.6	0.3
18:1	15.4	9.3
18:2	12.4	3.1
16:1 + 18:1/ 16 + 18	2.6	3.9
% Unsaturated	68.4	72.0

^a Cells were grown to late logarithmic phase at indicated growth temperature. Results are expressed as percent of total fatty-acid composition.

^b Less than 0.1% of total.

composition. The effect of lowered salt concentration on the growth of sediment isolate 3SE8 is illustrated in Fig. 8. Growth in the complete salts medium showed a rapid increase, with gradual slowing of the division rate after approximately 3 h. Growth in the one-half-strength medium also showed a rapid increase, but optical density readings were always lower than for cells grown in the complete salts medium. Cells grown in both salt concentrations were harvested after 7 h, with yields of 2.3 and 1.0 g of cells (wet weight), calculated on a 1-liter basis for complete and one-half-strength salts media. Cells grown in one-quarter- or one-eighth-strength salts media showed considerable reduction in growth rates. Cells grown in 1.4-strength salts medium were harvested after 22.5 h (optical density, 0.29), yielding 0.4 g of cells (wet weight) per liter. Cells in one-eighth-strength salts medium were not examined further. Cells inoculated into a distilled water-based medium showed no growth after

FIG. 7. Effect of growth temperature on the fatty-acid profile. Strain used was *Vibrio marinus* PS-207. See text for procedural details. A, 25 C; B, 15 C.

FIG. 8. Effect of reduced salt concentration on the growth of marine sediment isolate 3SE8.

22.5 h, as was expected, since the strain demonstrated a salt requirement for growth. The very slight increase in optical density that was noted was attributed to the limited increase in cell number supported by trace amounts of salts present in peptone and yeast extract (40, 54).

Light microscope examination of cells from one-half-strength salts medium showed no gross morphological differences compared with cells grown in complete medium. Cells from one-quarter-strength salts medium were irregular, with distended and pear-shaped cells observed. The pleomorphism was attributed to a weakening of the cell envelope caused by interruptions of the mucopeptide layer, as shown by MacLeod (40) for a marine pseudomonad in a medium with salt content lowered beyond the critical point.

Examination of fatty-acids obtained from lipids of cells grown in the complete, one-half- and one-quarter-strength salts media (Table 7) showed a marked difference in the unsaturated-to-saturated C16 + C18 ratios and in the percentage of total unsaturated fatty acids (see Fig. 9). Cells grown in the complete salts medium showed a 2:1 ratio of C16:1 to C16,

FIG. 9. Effect of reduced salt concentration on the fatty-acid profile of marine sediment isolate 3SE8.

whereas cells grown in a one-quarter-strength salts medium showed a ratio of almost 1:1 for the two acids. The most marked change in the fatty acids of cells grown in the three media was in C16:1 composition; i.e., a steady decrease in content was observed. At the same time, an almost equal increase in the saturated C16 acid was evident, although little difference was observed between growth in the one-half- and one-quarter-strength salts media. No change in either C18 or C18:1 was observed in cell extracts prepared from cells grown in any of the three media. The only other change of significance was in C14 and C14:1 content, both of which increased as the salt content was lowered.

DISCUSSION

The finding that C16:1 was the most prominent fatty acid of extracts prepared from 14 of the 22 strains studied is contrary to most reports on fatty acids of gram-negative bacteria. C16 is commonly the major species (13, 38, 43). The most likely explanation for this observation is in the growth temperature used here (25 C) as opposed to that (37 C) used in most of the studies reported in the literature. It is well established that lowering the growth temperature results in an increase in the percentage of unsaturated fatty acids (31, 43, 50). C16:1 can be the predominant fatty acid, as was shown by Blumer et al. (7) for marine and terrestrial ammonia-oxidizing bacteria, by Kates and Hagen (32) for a psychrophilic *Serratia* strain, and for *Photobacterium* by Cho and Salton (13). This characteristic may be an effect of growth temperature and not solely a feature common to marine bacteria in general since the C16:1 component was also prominent in the strain of *Vibrio cholerae*, a nonmarine species.

The presence of C18:2 in the cell extracts contradicts the generally accepted observation that polyunsaturated fatty acids do not occur among members of the order *Eubacteriales* (38, 43, 49). In pursuing this observation further, peptone and yeast-extract components of the medium used throughout the study were

TABLE 7. Effect of salt concentration on fatty acids of 3SE8^a

Fatty acid	Total fatty acids (%)		
	Complete medium	One-half-strength medium	One-quarter-strength medium
10	tr ^b	0.1	tr
11	tr	tr	0.3
12	4.0	0.7	4.0
i14	0.3	tr	0.2
14	3.0	5.2	6.1
14:1	0.4	1.0	1.3
15	1.7	1.7	1.2
i16	1.2	0.1	1.6
16	24.8	33.9	33.1
16:1	50.7	46.3	40.5
17	0.8	tr	0.7
17:1	1.4	tr	0.2
18	1.9	1.7	1.7
18:1	9.7	10.2	8.3
18:2	tr	tr	1.6
16:1 + 18:1/ 16 + 18	2.3	1.6	1.4
% Unsaturated	62.3	57.5	51.9

^a Cells were grown at 27 C in media containing various concentrations of salts (see Materials and Methods for details of media compositions). Results are expressed as percent total fatty-acid composition.

^b Less than 0.1% of total.

extracted and were found to contain the fatty acid C18:2. Thus, the strains examined in this study were able to incorporate C18:2 directly into phospholipids, or the acid may have been adsorbed to the cell surface. It is unlikely that adsorption occurred since the cells were washed three times with a salt solution composed of NaCl, KCl, and MgSO₄ to remove any contaminating medium from the cell surface. In addition, such a washing procedure is almost universally carried out before extraction of bacterial lipids. If the C18:2 component was adsorbed from the medium, its occurrence would have been reported in earlier studies of fatty acids of bacteria. Although it has generally been accepted that bacteria lack polyunsaturated fatty acids, several reports of polyunsaturated fatty acids in bacteria have been published. Huston and Albro (28) reported the occurrence of a C20:4 acid in extracts of *Sarcina lutea* amounting to 4% of the total fatty-acid composition. Hunter and James (27) reported a C18:2 among fatty acids of *Bacillus megaterium*, about 5% of the total acids. Tornabene et al. (51) reported C18:2 among the fatty acids of *Sarcina lutea* grown in Trypticase soy broth, but not when the cells were grown in nutrient broth. The presence of C18:2 in *S. lutea* was confirmed in another study using combined gas chromatography-mass spectroscopy (52). Cho and Salton (13) reported the occurrence of C18:2 among the fatty acids of *Aerobacter aerogenes*, in two strains of *Serratia marcescens*, and in an unidentified *Pseudomonas* sp. Yano et al. (55) studied the fatty acids of *Arthrobacter simplex* grown in a variety of hydrocarbon-based media and reported the presence of C18:2, to about 20% of the total fatty-acid composition, and confirmed their results by using mass spectroscopy. Romero and Brenner (47), in examining fatty acids of *Pseudomonas aeruginosa* grown with hexadecane as a sole carbon source, identified C18:1 and C18:2 as the major fatty acids, with the dienoic acid comprising over 35% of one of the lipid fractions. They also identified C18:2, C20:2, and C21:2 as excretory products in the growth medium.

That bacteria are capable of de novo synthesis of polyunsaturated fatty acids is evidenced by the work of Yano et al. (55) and Romero and Brenner (47), who showed conversion of hydrocarbons, used as sole carbon sources, to polyunsaturated fatty acids. The work of Cho and Salton (13) further suggests that simple adsorption of C18:2 from the medium does not explain its presence in the fatty acid extracts, since isolated cell envelopes were used.

Unfortunately, except for the brief discussions by Yano et al. (55) and Romero and Brenner (47) on the possible mode of synthesis of the C18:2 component, none of the published reports provides an explanation of the significance of C18:2. Furthermore, the presence of C18:2 among the fatty acids of various bacterial species is not even acknowledged by many workers. The presence of polyenoic acids, confirmed by mass spectroscopy or found in isolated bacterial envelopes, should be a matter of considerable significance. Because C18:2 was identified in the extracts of *Pseudomonas aeruginosa* ATCC 14216 (Table 2) as well as in other nonmarine bacteria, it would appear that this acid is not restricted to marine bacteria. It may be significant, however, that it was found to occur in only one of the non-marine strains examined yet was present in 15 of the 20 estuarine and marine isolates examined. Further studies on the incorporation or synthesis, or both, of C18:2 clearly are needed.

It is interesting to note that in the *Vibrio* strains examined, unsaturated-to-saturated C16 + C18 ratios were lowest (i.e., saturated acids were highest) in the marine strains. This is similar to the findings of Blumer et al. (7), who found higher degrees of saturatedness in the fatty acids of marine ammonia-oxidizers than in their non-marine counterparts. This was somewhat surprising, because lower temperatures associated with the marine environment suggest that a higher degree of unsaturated rather than saturated fatty acids should exist among the marine bacteria. A possible explanation may be that a growth temperature of 25 C was used in this study. Since over 80% of the ocean is 5 C or less (39), it can be assumed that a laboratory growth temperature of 25 C is relatively high and would induce marine bacteria to synthesize greater amounts of saturated acids. On the other hand, estuarine isolates, the temperature of the normal habitats of which may reach 40 C or more, and *Vibrio cholerae*, the optimal growth temperature of which is 37 C, would compensate for the low growth temperature of 25 C by production of a greater proportion of unsaturated acids. Kojima (34) showed that when the growth temperature was set at 37 C, an unsaturated-to-saturated C16 + C18 ratio of 1.4 was found for *Vibrio parahaemolyticus* and 1.1 to 1.9 for *V. cholerae*. A ratio of 1.35 was found for *V. cholerae* grown at 37 C by Brian and Gardner (8). It is well established (39, 46) that a lowered growth temperature causes increased synthesis of unsaturated acids. However, our data suggest that the optimal growth tempera-

ture of an organism should be considered when comparing fatty-acid profiles.

The fatty acids reported here for a number of *Vibrio* strains are in agreement with other published data (8, 13, 34). The increased amount of C16:1 observed to be present in the strains examined in this study can be attributed to the lower growth temperature of incubation.

The fatty-acid composition of the two *Pseudomonas* strains studied was found to be similar to that reported by Brian and Scholes (Bacteriol. Proc., p. 143, 1967) for *P. aeruginosa*, although, again, the unsaturated content was higher in this study, most probably because of the differences in growth temperature used (25 versus 37 C). Hancock and Meadow (23) reported the occurrence of cyclic C19 in *P. aeruginosa*, about 38% of the total composition, when the cells were harvested from the stationary phase. The formation of the cyclic acid was due to a conversion of C18:1, a phenomenon observed by other workers as well (18, 43). No cyclic acids were found in extracts prepared from either *P. aeruginosa* ATCC 14216 or *P. perfectum* ATCC 14405, but strain differences or the short incubation period used may be involved. The C18:1 content (47%) reported by Hancock and Meadow (23) for *P. aeruginosa* harvested from the logarithmic phase was, however, almost identical to that found for the two pseudomonads examined in this study.

The most distinguishing feature of *Arthrobacter marinus* ATCC 25374 was the presence of cyclic C17. Cyclic acids have been reported in a variety of bacterial genera, including *Escherichia*, *Agrobacterium*, *Serratia*, *Pseudomonas*, *Pasteurella*, *Salmonella*, *Aerobacter*, *Vibrio*, and *Lactobacillus* (5, 8, 12, 23, 25, 30, 31, 36). Trace amounts of cyclic C19 may have been present in the extracts of *A. marinus* ATCC 25374, but this acid was not detected; both fatty acids might have been found in greater concentrations if a longer incubation time (19, 23, 31, 43) or a higher growth temperature had been used (31, 33). The few reports available on the fatty acids of *Arthrobacter* spp. have indicated a composition similar to that of the gram-positive bacteria, with branched acids as the predominant form. Shaw and Stead (48) examined the fatty acids of three terrestrial species of *Arthrobacter* and reported two *anteiso*-branched acids to comprise over 94% of the total composition. Kostiw et al. (35), reporting on the membrane lipids of growing and starving cells of *A. crystallopoietes*, noted a similar fatty-acid pattern in the cells of both growth stages, with the *anteiso* acids C15 and C17 comprising 88% of the total

acids. Similar results have been reported by Yano et al. (55) for *A. simplex*. It is evident that the fatty acids of *A. marinus* ATCC 25374, the type strain of the species, are in no way similar to those of strains of other species of this genus and are, in fact, quite typical of gram-negative bacteria in general. On the basis of the phospholipid composition, the taxonomic data (6, 44), and the fatty-acid profiles reported here, it is concluded that *A. marinus* should be removed from the genus *Arthrobacter*. Baumann et al. (6) have suggested placement of this organism in the genus *Pseudomonas*.

The percentage of C18:1 in *Agrobacterium stellulatum* ATCC 15215 (>93% of total acids) served to differentiate this strain readily from the other strains examined. There was a lack of cyclic fatty acids in extracts of this marine isolate, yet these have been reported for *A. tumefaciens* (25, 26, 30) and *A. radiobacter* (21). Marr and Ingraham (41) noted, however, that in *E. coli* the formation of cyclic C17 was very minor at low growth temperatures, being formed in substantial amounts only at a temperature of 43 C. Effect of age was also demonstrated, and a 12-fold increase in the cyclic C17 concentration was shown to occur during the stationary phase compared with cells harvested during the logarithmic phase. Similarly, cyclic C19 could only be detected in cells harvested at the stationary phase. If the same effects are operating for members of the genus *Agrobacterium*, then it must be assumed that the parent unsaturated acids (C16:1 and C18:1) would still be represented in significant quantities until the cyclic acids are formed. Our finding that 93% of the total acids were represented by C18:1 suggested that this was a plausible assumption. If the amounts of C18:1 and cyclic C19 acids found by both Hildebrand and Law (25) and Kaneshiro and Marr (30) are summed, totals of 84 and 77%, respectively, are obtained from the two studies. These amounts are in the range reported here for C18:1. The total of 91% unsaturated fatty acids (including cyclic acids) reported by Hildebrand and Law is very close to the results of this study, using *A. stellulatum* ATCC 15215. Other than the C18:1 and cyclic C19 fatty acids reported for *A. tumefaciens* (25), the amounts of the acids remaining were quite similar to those found here for *A. stellulatum* ATCC 15215.

The only report concerning the fatty acids of *Achromobacter* spp. is a note by Hardy et al. (Proc. Soc. Gen. Microbiol. 5:iii), who stated only that the major fatty acids found were C18:1 and C16:1 in two closely related species isolated from Atlantic salmon. Whereas the

overall fatty-acid composition appeared to be, in general, similar to that of the other organisms examined, low concentrations of C16 and C16:1 and a moderately high concentration of C18:1 served to differentiate this genus from the other genera studied.

The similarity in fatty-acid profiles of *Spirillum linum* ATCC 11336 and of the vibrios might be expected, since both are members of the same family (*Spirillaceae*). This is the first report on the fatty-acid composition of a *Spirillum* strain, and it is, therefore, impossible to compare this result with data for strains of other species of this genus. Based on the close similarity of *S. linum* to the vibrios examined, however, it appears that the relatively close taxonomic relationship of these two genera is justified on the basis of fatty-acid composition.

Other than the finding of higher amounts of C14, trace amounts of C18, and a rather high concentration of C18:2, the fatty-acid composition of *Photobacterium fischeri* ATCC 7744 was observed to be similar to that of the vibrios examined. Such a similarity in fatty-acid composition might be expected, as the phospholipid pattern was found to be similar (44), and a close taxonomic relationship between *P. fischeri* and *Vibrio* spp. has been observed (16, 17). The deoxyribonucleic acid base composition data obtained for strains of *Photobacterium* spp. (15, 17) indicate that the genus is an artificial construct based on the single characteristic of luminescence. It is proposed that *Photobacterium fischeri* be placed in the genus *Vibrio*, as suggested by Lehmann and Neumann in 1896 (37). The fatty-acid composition determined for *P. fischeri* ATCC 7744 was similar to that reported for *P. albensis* by Cho and Salton (13), the only other report available on members of this genus. No component corresponding to that tentatively identified as an unsaturated C9 in *P. fischeri* ATCC 7744 was identified in the luminescent estuarine isolate 1ORR10.

As with phospholipids of the eight sediment isolates (44), the fatty acids of these bacteria revealed a wide range in the quantitative fatty-acid patterns, although qualitatively the isolates were similar. The patterns observed were, for the most part, similar to those of the reference *Vibrio* strains examined. Such close similarities might be expected, since from the deoxyribonucleic acid base composition analyses and the morphological and biochemical data, all but two of the isolates (strains 3S2 and 3SE5) were identified as belonging to species of the genus *Vibrio* (44).

Isolate 3SE5 was similar, with respect to fatty-acid composition, to the gram-positive

bacteria which are known to contain considerable amounts of branched, but little unsaturated, fatty acids (13, 38, 43). Despite these similarities, 3SE5 is a gram-negative bacterium. Other comparable situations have been reported (e.g., the fatty acids of the extreme thermophile *Thermus aquaticus* and of *Escherichia alcalescens*). Ray et al. (46) reported the major fatty acid of *T. aquaticus* to be an *iso*-branched C15 (35-56%), with the second most abundant species being an *iso*-branched C15 (28-33%). Cho and Salton (13) reported a branched C17 acid to be the second most abundant component (26.4%) in the fatty acids of *E. alcalescens*, with C16 predominant (46%). The lipids of isolate 3SE5 appeared to be similar to those typical of gram-negative bacteria, i.e., they lacked the glycolipids or phospholipids characteristic of gram-positive microorganisms.

The increases observed in the C16:1 content of marine sediment isolate 3SE8 with increased culture age were contrary to the findings reported in the literature for nonmarine microorganisms (43). Kates (31) and Marr and Ingraham (41) noted a decrease in the C16:1 content of *E. coli* with increased culture age, but the decrease was paralleled with an increase in the cyclic C17 concentration. A similar observation was cited for *Lactobacillus*, where a conversion of C18:1 to cyclic C19 (53) was noted. Also, *P. aeruginosa* showed a conversion of both C16:1 and C18:1 to cyclic C17 and C19 (23). No cyclic fatty acids were found to occur at any point in the growth of strain 3SE8, thus explaining the lack of conversion of the unsaturated species to the cyclic acids. The increased C16:1 content may be correlated with the large increases in phosphatidylethanolamine observed for this isolate with increased culture age (44). The increase in the C16:1 component did not appear to be characteristic of the marine isolates, since isolate 3SE7 showed only a slight increase and 3S2 demonstrated a slight decrease. Whether the age effect on C16:1 content was due to growth temperature or to medium used or was a feature of the organisms themselves is not known.

Lowering the growth temperature of *Vibrio marinus* strain PS-207 from 25 to 15°C appeared to have an effect on fatty-acid composition similar to that reported for many nonmarine bacteria. No qualitative differences were found and no chain-length reduction was evident, with the effect of lowered temperature reflected primarily in an increase in C16:1. A mechanism as yet unelucidated may account for the selective increase in the unsaturated components, as shown by Okuyama (42).

It is interesting to compare the fatty-acid

composition of *V. marinus* strain PS-207, grown at 15 C, with that of the obligately psychrophilic strain *V. marinus* MP-1 reported by Oró et al. (45). The fatty-acid composition of strain MP-1 was examined after harvesting the cells from the late logarithmic phase of growth. The major acids found were C16:1 (31.1%), C18:1 (22.8%), C16 (19.6%), and C14 (14.4%), with an unsaturated-to-saturated C16 + C18 ratio of 2.5. This is quite different from that of strain PS-207, where C16:1 amounted to 57.5% and C18:1 to only 9.3%. When, on the other hand, the pattern found for strain PS-207 grown at 25 C is compared with strain MP-1 grown at 15 C, the percentages of the major acids are similar, with C16:1 representing only 39.2% and C18:1 amounting to 15.4%, with an unsaturated-to-saturated C16 + C18 ratio of 2.6. It is clear that comparisons of fatty-acid composition among bacterial strains should be done by using extracts of cells grown at the temperature optimum for each of the strains tested. The optimal growth temperature is 25 C for strain PS-207 and 15 C for MP-1. Attempts to compare extracts of strains grown at 15 C may well lead to erroneous conclusions. Growth of both strains at their temperature optima revealed similar fatty-acid profiles.

The effect of lowered salt concentration in the growth medium on the fatty acids of marine bacteria may be attributed to a weakening of the cell membrane, i.e., lack of screening of the electro-negative charges of the mucopeptide layer by the divalent salt cations (40). Similar findings were reported by Brown (11), who studied the effects of salt concentration on the cell envelope of a marine pseudomonad and found that cells grown in salt concentrations of 0.98 to 0.25 M (equivalent to complete and one-half-strength salts media of this study), were "normal," whereas those grown in media containing less than 0.18 M salts (the one-quarter- and one-eighth-strength salts media of this study contained 0.11 and 0.055 M salts, respectively) were short and somewhat rounded and gave reduced cell yields. The requirement for multivalent cations has been well documented for marine bacteria (40, 41). The possibility that marine microorganisms may not tolerate salt concentrations significantly lower than that of the open ocean (as, for example, in the estuary) is an interesting factor to be considered (S. O. Stanley and R. Y. Morita, *Bacteriol. Proc.*, p. 45, 1967).

Cells grown in complete and one-half-strength salts media were harvested after 7 h of growth, and those from one-quarter-strength

salts medium were harvested after 22.5 h. Changes in C16 and C16:1 components that were noted may be due to increased age of the culture. O'Leary (43) noted that, in general, the percentage of saturated fatty acids increased with the age of the cultures. Results given in Table 3, however, showed little change in the percentage of either C16 or C16:1 over a comparable growth period. It must, therefore, be concluded that salt concentration effected the differences observed in C16 and C16:1 fatty-acid content.

In conclusion, the fatty-acid profiles of the marine bacteria examined in this study were found to be similar to those of non-marine species. Some of the fatty acids of these microorganisms were noted and considered to be significant. Of the variety of fatty acids found to occur among the strains, C16:1 was dominant. In 14 of 22 strains, C16:1 was the predominant fatty acid, and in the other strains studied it was the second predominant species in all but the strain of *Agrobacterium stellulatum*. In our investigation of the effect of culture age on fatty-acid profiles, C16:1 was the only acid found to change significantly with increased time of growth. The same effect was observed for fatty acids of *V. marinus* strain PS-207, with C16:1 increasing significantly when the growth temperature was lowered from 25 to 15 C. Lowering the salt concentration caused the C16:1 content of bacterial isolate 3SE8 to be markedly reduced, with a compensatory increase noted in C16. The presence of C18:2 was found to be significant, since it was detected in extracts of 15 of the 20 marine and estuarine strains studied.

Growth temperature was found to be important in interpreting fatty-acid profiles of bacteria. Evidence obtained from this study suggests clearly that comparisons can be made only when organisms are grown at their temperature optima. The *Vibrio* strains examined were easily differentiated from the strains of other genera studied. The taxonomic position of *Arthrobacter marinus* must be reevaluated, and *Photobacterium fischeri* should be reassigned to the genus *Vibrio*, where it was placed by Lehmann and Neumann in 1896 (37).

Marine sediment isolates were not uniform in their fatty-acid profiles, although most of the isolates could be identified as belonging to the genus *Vibrio* on the basis of fatty-acid composition. Sediment isolate 3SE5 demonstrated a rather unique pattern wherein branched acids comprised the major class of fatty acids.

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REPRINT REQUESTS

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