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Chemotaxonomic Classification of Marine Bacteria on the Basis of Fatty Acid Compositions

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The cellular fatty acids of 47 marine bacteria representing the genus *Alteromonas, Arthrobacter, Bacillus, Micrococcus, Pseudomonas, Shewanella, Staphylococcus* and *Stenotrophomonas* were determined by a gas-liquid chromatographic analysis. Sixty-eight different fatty acids with 10 to 20 carbon atoms were detected in marine bacteria. Of the eight genus examined, 14:0, 16:0 and i17:0 were detected in all, while i14:0, a15:0, i16:0, and 15:0 were found in most of all. There were significant differences in the fatty acid patterns between Gram positive and Gram negative bacteria. Bacteria of Gram positive genus showed relatively high contents of the branched type fatty acids, while the major fatty acids in Gram negative were unsaturated and straight forms. Phylogenetic relationships between marine bacteria defined by the cellular fatty acid patterns represented obvious differences between Gram positive and Gram negative genera, even in respective genus. Therefore, the bacterial classification and identification can be accomplished more easily and rapidly based on the cellular fatty acid profiles than the conventional methods.

Key words : cellular fatty acid, chemotoxanomy, marine bacteria, phylogenetic relationship

Introduction

Fatty acids which are important structural component occur in the cell membranes of bacteria as the acyl constituents of phospholipids (Katayama-Fujimura et al., 1982). Majority of marine bacteria have the fatty acid of 12~20 carbon chain-length, which are characterized by the ubiquitous even-chain saturated and *cis*-monounsaturated acids as well as odd-chain and the branched acids, and hydroxy and cyclopropyl derivatives (Shaw, 1974; Tornabene, 1985; Padley et al., 1994).

The fatty acid compositions of bacteria in the ocean may vary considerably with changes in environment conditions or growth phase such as nutrient, salt, temperature and toxic substance (Guckert et al., 1986; Hanna et al., 1984; Nichols et al., 1994; Weber et al., 1994; Kieft et al., 1997). However, if the bacteria grown under standardized conditions, they may have a stable fatty acid composition which is specific to a genus or even a species (Miller and Berger, 1985). It was reported that the shift in fatty acid composition induced by changes in growth conditions did not seriously affect the phylogenetic relationship analysis by phospholipid acids (Kohring et al., 1994).

The cellular fatty acids can be extracted and measured with relatively simple methods (Bligh and Dyer, 1959; White et al., 1979), and the identification of bacteria by their fatty acid compositions showed in good agreement with genomic hybridization data (Fulco, 1983; Guckert et al., 1991; Kohring et al., 1994). Therefore, the cellular fatty acid analysis are widely used currently as a valuable chemotaxonomic tool for the classification and identification of marine bacteria (Seong et al., 1992; Kohring et al., 1994; Bertone et al., 1996; Kang et al., 1997).

As a part of surveying the ecological and biochemical aspects of marine bacteria, we examined the biochemical characteristics and phylogenetic relationship of marine bacteria based on the patterns of cellular fatty acids from isolates recovered from coastal waters.

Materials and Methods

Strains and culture conditions

All of the test strains studied were isolated in Suyeong Bay (Kang et al., 1997). For the cellular fatty acid analysis, a total of 47 strains (Table 1) were subcultured onto TSA (trypticase soy agar) medium and incubated

Table 1. List of marine bactria used for the study (Kang et al., 1997)

Species		Strain number
Alteromonas	*haloplanktis*	SU12, SU165
Arthrobacter	*globiformis*	SU174
	oxidans	SU37
	protophormiae	SU20, SU162, SU191
	viscosus	SU24
Bacillus	*circulans*	SU57
	laterosporus	SU147
	licheniformis	SU9032, SU177
	megaterium	SU158, SU167, SU175, SU182
	mycoides	SU157
	psychrophilus	SU190
	pumilus	SU22, SU23, SU25, SU36, SU38, SU9022, SU9036
	sphaericus	SU145
	subtilis	SU16, SU149, SU163, SU171, SU176
Micrococcus	*lylae*	SU9027, SU156, SU179
	varians	SU168, SU180
Pseudomonas	*stutzeri*	SU11, SU14
	vesicularis	SU154
Shewanella	*putrefaciens*	SU148, SU153
Staphylococcus	*aureus*	SU90, SU140
	cohnii	SU184
Stenotrophomonas	*campestris*	SU152
	maltophilia	SUPM, SUPM1

at 28℃ for 24 hours until the beginning of the stationary phase. Then the bacteria were collected by centrifugation at about 4,000×*g* for 20 min and washed twice with deionized water.

Fatty acid analysis

Total cellular fatty acids were analyzed using the Microbial Identification (MIDI; Newark, Delaware) system by the standardized procedure described by Miller and Berger (1985). Approximately 40 to 50 mg of concentrated whole cell was incubated for 30 min at 100℃ after addition of 1 mℓ of 15% (wt/vol) NaOH in 50% aqueous methanol. The samples were then acidified to pH 2 by adding 6N HCl in CH_3OH, and the methylated fatty acids were further extracted with 1.25 mℓ of a 1:1 (vol/vol) solution of methyl-*tert*-buthyl ether-hexane. The organic extract was washed with 3 mℓ of 1.2% (wt/vol) NaOH, and centrifugated at about 900×*g* for 3 min.

Fatty acid methyl esters (FAMEs) were analysed by gas-liquid chromatography on an HP 5890A gas chromatograph (Hewlett-Packard Co. USA) equipped with a flame ionization detector. A fused-silica capillary column (0.2 mm× 25 m; cross-linked 5% methyl phenyl silicone [Hewlett-Packard Co. USA]) with ultrahigh-purity hydrogen as the carrier gas was used. The details of the gas-liquid chromatography conditions are as follows: injector temperature, 250 ℃; detector temperature, 300℃; initial column temperature, 170℃, increasing by 5℃/min to 270℃ in 20 min; carrier gas flow rate, 50℃/min; sample volume, 1μℓ. The peak retention time and peak area values were recorded with an HP 3392A integrator (Hewlett-Packard Co. USA). FAMEs were calibrated against a standard mixture of known fatty acids provided by MIDI. Detected sample peaks were named by interpolation of retention time using the equivalent chain length method. The result were expressed as percentages relative to the total peak area.

Fatty acid nomenclature

Fatty acids were designated as A:Bω C, where A is the total number of carbon atoms, B is the number of double bonds and C is the double bond from the aliphatic (ω) end of the molecule. Double bond geometry is indicated as 'c' for *cis* or 't' for *trans*. In addition, the prefix 'i' and 'a' denote *iso*- and *anteiso*-methyl branching, respectively; the locations of hydroxy (OH), or cyclopropane (cyc or cyclo) groups were also noted. Summed-in-feature values (SIFs) represent groups of fatty acids which could not be separated by gas-liquid chromatography with the MIDI system; SIF 2=15:1 *iso* H and/or 13:0 3OH; SIF 3=16:1 *iso* I and/or 14:0 3OH; SIF 4=15:0 *iso* 2OH and/or 16:1 trans 7; SIF 5=18:1 *iso* and/or *anteiso*; SIF 7=18:1 *cis* 7, 18:1 *trans* 9 and/or 18:1 *trans* 12.

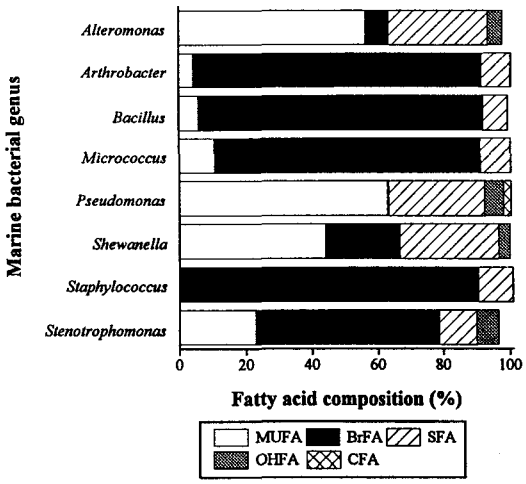

Fig. 1. Compositions of cellular fatty acid detected from marine bacteria.
MUFA: monounsaturated fatty acid, BrFA: branched fatty acid, SFA: saturated fatty acid, OHFA: hydroxy fatty acid, CFA: cyclopropane fatty acid

Results

Sixty-eight different fatty acids with 10 to 20 carbon atoms were detected in marine bacteria and were used in the statistical analysis. Mean percentage, standard deviations and ranges for each genus are listed in Table 2. The fatty acids that were found less than 1% in all the strains or less than 3 of each marine genus are not included. Straight fatty acids of 14:0 and 16:0 were found from all of the tested marine bacterial genera but branched i17:0, however i14:0, a15:0 and i16:0 were not found in the genus *Pseudomonas*, and 15:0 was not detected in the genus *Staphylococcus* (Table 2). In addition, acids of less than C14 occurred only in very small amounts, and no fatty acids of greater than 20 carbons were present.

Among the marine bacterial strains studied, branched fatty acids were predominant in Gram positive genera (*Arthrobacter, Bacillus, Micrococcus and Staphylococcus*), while unsaturated and straight forms were detected in Gram negative bacteria (*Alteromonas, Pseudomonas, Shewanella* and *Stenotrophomonas*), with the exception of the genus *Stenotrophomonas* (Fig. 1). As expected for the fatty acid profiles of marine bacteria, straight acids had preponderantly even carbon number, especially 16:0 and 18:0 (Table 3).

As shown in Table 2, the genus *Arthrobacter* possessed 21 kinds of fatty acids, with a predominance of a15:0 (56.25%). Moderate quantities of i15:0 (10.38 %), and of i16:0, a17:0 and 16:0 were found as well (7.59 %, 5.48% and 5.40%, respectively). From the genus *Bacillus*, 21 different fatty acids were detected. The predominant acids were i15:0 and a15:0 (43.21% and 29. 02%, respectively). From the genus *Micrococcus*, 19 different fatty acids were found. The major fatty acid was a15:0 (41.85%) followed by i15:0 (23.24%), a17:0 (6.86%), and 16:0 (5.52%). From the genus *Staphylococcus*, 10 different fatty acids were detected. The preponderant one was a15:0 (46.63%), and abundant amounts of i15:0 (14.14%) and a17:0 (13.02%) were found. Small amounts of i17:0 (6.81%) and 20:0 (3.65%) were found as well. Among the acids extracted from this genus, characteristic feature was the content of unsaturated fatty acids which weren't detected at all from the other Gram positive genera.

Among Gram negative bacteria, the genus *Alteromonas* contained 24 fatty acids. The most abundant fatty acids were 16:1ω7c, 16:0 and 17:1ω8c (61.27% in all) followed by 17:0 (5.38%). Among the 13 fatty acids found in the genus *Pseudomonas*, SIF 7 was the major acid, with 16:0 and 16:1ω7c being the second and the third most prominent, and these three major acids were

Table 2. FAME compositions of the marine bacterial genera studied

Genus	Percentage of following fatty acid in genus:																													
	12:0	11:0 *iso* 3OH	13:0 *iso*	13:0 *anteiso*	12:0 3OH	14:0 *iso*	14:0	SIF2	15:0 *iso*	15:0 *anteiso*	15:1 *cis*8	15:1 *cis*6	15:0	16:1 *cis*7 alcohol	SIF2	16:0 *iso*	16:1 *cis*11	16:1 *cis*9	16:1 cis7	SIF3	16:0	SIF5	17:0 *iso*	17:0 *anteiso*	17:1 *cis*8	17:0	18:1 *cis*9	SIF7	18:0	20:0
Alteromonas																														
Mean	0.80	0.22	0.00	0.00	3.00	0.10	0.90	1.30	0.07	0.25	4.28	0.41	4.30	0.00	0.07	1.60	0.00	0.46	29.50	3.50	16.55	0.00	0.36	0.72	15.22	5.38	0.07	3.95	0.52	0.00
SM	0.80	0.22	0.00	0.00	3.00	0.10	0.04	0.70	0.07	0.05	0.71	0.08	0.18	0.00	0.07	0.29	0.00	0.46	1.30	3.50	1.20	0.00	0.11	0.03	2.88	1.16	0.07	3.95	0.14	0.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.60	0.00	0.20	3.57	0.33	4.12	0.00	0.00	1.31	0.00	0.00	28.19	0.00	15.35	0.00	0.25	0.69	12.34	4.22	0.00	0.00	0.38	0.00
Maximum	1.59	0.44	0.00	0.00	6.00	0.19	0.94	1.99	0.13	0.29	4.99	0.49	4.48	0.00	0.14	1.88	0.00	0.92	30.80	7.00	17.35	0.00	0.46	0.75	18.09	6.54	0.13	7.89	0.64	0.00
Arthrobacter																														
Mean	0.00	0.19	0.04	0.23	0.00	4.94	2.23	0.11	10.38	56.25	0.00	0.03	0.69	0.48	0.27	7.59	0.98	0.00	0.33	1.69	5.40	0.16	0.67	5.48	0.00	0.00	0.00	0.00	0.19	0.00
SD	0.00	0.41	0.09	0.26	0.00	2.11	0.62	0.24	7.93	18.30	0.00	0.07	0.61	1.06	0.61	2.95	2.18	0.00	0.52	3.77	2.16	0.35	0.75	1.80	0.00	0.00	0.00	0.00	0.42	0.00
Minimum	0.00	0.00	0.00	0.00	0.00	1.60	1.21	0.00	3.31	20.79	0.00	0.00	0.00	0.00	0.00	3.32	0.00	0.00	0.00	0.00	1.81	0.00	0.00	2.16	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.00	1.11	0.25	0.72	0.00	8.70	3.05	0.64	26.11	71.23	0.00	0.20	1.76	2.85	1.63	12.18	5.85	0.00	1.39	10.12	8.05	0.95	1.73	7.18	0.00	0.00	0.00	0.00	1.14	0.00
Bacillus																														
Mean	0.00	0.02	0.49	0.06	0.00	2.08	2.07	0.00	43.21	29.02	0.00	0.00	0.16	1.01	0.10	2.37	1.46	0.00	0.00	0.45	4.51	0.61	4.58	4.02	0.00	0.11	0.11	0.00	0.33	0.30
SD	0.00	0.09	1.32	0.29	0.00	1.94	2.96	0.00	12.58	10.04	0.00	0.00	0.71	2.06	0.45	2.02	1.09	0.00	0.00	2.13	4.99	1.16	3.12	3.09	0.00	0.41	0.23	0.00	0.81	1.40
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.17	3.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.00	0.42	6.47	1.35	0.00	7.53	13.56	0.00	63.58	47.72	0.00	0.00	3.34	9.01	2.20	8.63	3.86	0.00	0.00	10.43	23.73	0.95	10.27	12.57	0.00	1.94	0.69	0.00	3.56	6.87
Micrococcus																														
Mean	0.00	0.00	0.48	1.06	0.00	2.95	3.04	0.64	23.24	41.85	0.00	0.32	0.45	1.04	0.00	3.56	4.13	0.00	1.02	0.00	5.52	0.61	0.55	6.86	0.00	0.00	0.07	0.00	0.00	0.03
SD	0.00	0.00	0.65	1.48	0.00	1.32	2.92	1.28	16.91	20.48	0.00	0.64	0.89	1.49	0.00	2.65	7.34	0.00	2.04	0.00	3.71	1.16	0.58	7.41	0.00	0.00	0.14	0.00	0.00	0.06
Minimum	0.00	0.00	0.00	0.00	0.00	1.23	0.00	0.00	4.07	4.45	0.00	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00	0.00	1.05	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.00	0.00	1.63	3.77	0.00	5.21	8.17	3.20	48.68	58.29	0.00	1.59	2.23	3.82	0.00	8.16	18.74	0.00	5.11	0.00	11.67	4.56	1.56	16.85	0.00	0.00	0.34	0.00	0.00	0.15
Pseudomonas																														
Mean	4.95	0.00	0.00	0.00	3.51	0.00	2.02	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	19.37	0.00	21.17	0.69	0.36	0.00	0.31	0.11	0.08	42.91	0.54	0.00
SD	3.50	0.00	0.00	0.00	0.53	0.00	1.19	0.00	0.00	0.00	0.00	0.00	0.57	0.00	0.00	0.00	0.00	0.00	6.99	0.00	1.84	1.38	0.26	0.00	0.43	0.16	0.11	10.75	0.06	0.00
Minimum	0.00	0.00	0.00	0.00	2.80	0.00	1.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.55	0.00	18.68	0.00	0.00	0.00	0.00	0.00	0.00	32.20	0.46	10.00
Maximum	7.45	0.00	0.00	0.00	4.05	0.00	3.70	0.00	0.00	0.00	0.00	0.00	1.21	0.00	0.00	0.00	0.00	0.00	25.27	0.00	23.09	3.44	0.57	0.00	0.92	0.33	0.24	57.61	0.60	0.00
Shewanella																														
Mean	2.10	0.00	3.15	0.00	1.27	0.92	2.00	1.85	17.37	0.20	1.88	0.95	10.72	0.00	1.49	0.39	0.00	1.08	15.32	0.00	9.24	0.00	0.63	0.00	17.07	2.95	1.15	1.96	0.34	0.00
SD	0.06	0.00	0.25	0.00	0.04	0.12	0.06	0.21	1.81	0.01	0.37	0.13	0.79	0.00	0.11	0.00	0.00	0.07	0.09	0.00	0.83	0.00	0.16	0.00	1.55	0.30	0.15	0.07	0.07	0.00
Minimum	2.04	0.00	2.89	0.00	1.23	0.80	1.93	1.63	15.56	0.18	1.51	0.82	9.93	0.00	1.37	0.38	0.00	1.01	15.23	0.00	8.41	0.00	0.47	0.00	15.52	2.65	1.00	1.89	0.27	0.00
Maximum	2.15	0.00	3.40	0.00	1.31	1.04	2.06	2.06	19.18	0.21	2.24	1.08	11.50	0.00	1.60	0.39	0.00	1.15	15.41	0.00	10.06	0.00	0.78	0.00	18.62	3.24	1.29	2.03	0.41	0.00
Staphylococcus																														
Mean	0.00	0.00	0.00	0.00	0.00	1.67	0.31	0.00	14.14	46.43	0.00	0.00	0.00	0.00	0.00	3.27	0.00	0.00	0.00	0.00	2.22	0.00	6.81	13.02	0.00	0.00	0.00	0.00	4.12	3.65
SD	0.00	0.00	0.00	0.00	0.00	0.41	0.25	0.00	4.60	4.63	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.40	0.00	1.72	3.80	0.00	0.00	0.00	0.00	0.88	1.74
Minimum	0.00	0.00	0.00	0.00	0.00	1.35	0.00	0.00	10.40	40.36	0.00	0.00	0.00	0.00	0.00	2.77	0.00	0.00	0.00	0.00	1.68	0.00	4.48	7.69	0.00	0.00	0.00	0.00	3.18	1.80
Maximum	0.00	0.00	0.00	0.00	0.00	2.24	0.60	0.00	20.61	51.58	0.00	0.00	0.00	0.00	0.00	3.95	0.00	0.00	0.00	0.00	2.65	0.00	8.58	16.21	0.00	0.00	0.00	0.00	5.30	5.98
Stenotrophomonas																														
Mean	0.00	2.32	0.84	0.11	2.20	3.47	4.07	0.00	35.72	6.35	0.09	0.00	0.94	0.10	0.00	2.57	0.00	2.68	10.09	0.00	5.93	0.00	2.08	0.00	0.26	0.00	0.68	0.33	0.00	0.00
SD	0.00	0.83	0.17	0.15	0.84	3.94	0.44	0.00	4.64	1.71	0.13	0.14	0.00	0.34	0.14	0.00	0.90	0.90	0.82	0.00	0.43	0.00	0.73	0.00	0.23	0.00	0.50	0.25	0.00	0.00
Minimum	0.00	1.55	0.64	0.00	1.04	0.65	3.45	0.00	29.17	3.95	0.00	0.00	0.65	0.00	0.00	0.78	0.00	1.42	9.02	0.00	5.57	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	0.00	3.47	1.06	0.32	3.03	9.04	4.42	0.00	39.31	7.73	0.27	0.00	1.48	0.29	0.00	6.02	0.00	3.45	11.02	0.00	6.53	0.00	2.95	0.00	0.55	0.00	1.19	0.59	0.00	0.00

Table 3. The cellular fatty acid types of each marine bacterial genus studied

Genus	Unsaturated chain			Saturated chain																				Others[1]	Total
				Branched chain										Straight chain											
				Anteiso			Odd-iso				Even-iso			Normal											
	15	16	17	13	15	17	19	11	13	15	17	19	14	16	18	10	12	13	14	15	16	17	18	20	
Alteromonas	5.99[2]	30.03	16.34	4.02	–[3]	tr[4]	tr	–	–	–	3.57	tr	–	tr	1.60	tr	tr	1.22	tr	tr	4.30	16.55	5.38	tr	–	4.28	97.76
Arthrobacter	tr	3.12	1.03	–	tr	56.25	5.48	–	–	tr	12.07	tr	–	4.94	7.59	–	–	–	–	2.23	tr	5.52	–	tr	–	tr	100.52
Bacillus	tr	3.58	2.21	tr	tr	29.02	4.02	–	–	tr	43.66	4.58	–	2.08	2.37	tr	–	–	–	2.07	tr	4.31	tr	tr	tr	tr	99.67
Micrococcus	0.96	7.49	2.20	tr	1.06	41.85	6.86	–	–	tr	23.24	tr	–	2.95	3.56	–	–	–	–	3.04	tr	5.52	–	–	tr	tr	100.35
Pseudomonas	–	19.37	tr	42.99	–	–	–	–	–	–	–	tr	–	–	–	–	4.95	–	2.02	tr	21.17	tr	tr	–	7.78	100.31
Shewanella	4.68	17.89	18.38	3.11	–	tr	–	–	–	3.15	17.37	tr	–	tr	tr	–	–	2.10	2.25	2.00	10.72	9.24	2.95	tr	–	3.42	100.07
Staphylococcus	–	–	–	–	–	–	–	–	–	–	14.14	6.81	2.26	1.67	3.27	tr	–	–	–	tr	–	2.22	–	4.12	3.65	–	100.88
Stenotrophomonas	2.69	12.87	6.38	1.01	tr	6.35	–	tr	4.15	tr	35.72	2.08	tr	3.47	2.57	–	tr	–	–	4.07	tr	5.93	–	–	–	6.48	96.61

[1]Others: hydroxy and/or cyclopropane fatty acid, [2]all data indicate mean value for each marine genus, [3]–: not detected, [4]tr: less than 1.00%

>83% of totals. Compared to the other genus examined, this genus also contained a significant amount of cyclopropanate (2.22%). The genus *Shewanella* possessed 22 fatty acids, with a predominance of i15:0, 17:1ω8c, and 16:1ω7c (17.37%, 17.07% and 15.32%, respectively). Moderate quantities of 15:0 (10.72%) and 16:0 (9.24%) were also found. The one exception to the general fatty acid pattern observed among Gram negative bacteria was the genus *Stenotrophomonas* (formally *Xanthomonas*), which possessed 19 different acids. Among the acids detected from this genus, branched acids was the most abundant fatty acid group (55.71%). The main acid component was an *iso*-branched C_{15}, constituting almost 36% of the totals, with a15:0 also occurring in significant quantity (6.35%). 16:1ω7c (10.09%) and 16:0 (5.93%) were also found the major fatty acid in the genus *Stenotrophomonas*.

Discussion

All of the tested marine bacterial genera contained the fatty acids of 10 to 20 carbon atoms, which are commonly found in most of marine bacteria (Shaw, 1974). For all of marine bacteria examined in this study, unsaturated fatty acids were only in monoenoic form. This result is consistent with the report that bacteria generally do not contain polyunsaturated acids (Lechevalier and Lechevalier, 1988). Monoenoates in bacteria are known to be produced by the aerobic pathway, in which desaturases yield a variety of positional isomers which can be used in the classification of the source organisms (O'Leary and Wilkinson, 1988).

It was known, with respect to fatty acid composition, that Gram positive bacteria contain a high proportion of branched (*iso* and/or *anteiso*) and a little unsaturated fatty acids (O'Leary and Wilkinson, 1988; Kaneda, 1991). Cho and Salton (1966) also reported that Gram positive bacteria contained a high proportion of both C_{15} and C_{17} branched chain fatty acids. These features are in good agreement with Gram positive genera of this study, with more than 80% branched, especially C_{15} and C_{17} amounting for 72 to 81% of total, of fatty acid totals.

However, more branched fatty acids (>55% of total acids) was detected in the Gram negative bacteria *Steno-*

trophomonas. Branched chain fatty acids have been proposed to increase the fluidity of membrane (Kaneda, 1991). Fluidity can also be increased by decreasing the average chain length of the fatty acyl groups, or by increasing the ratio of unsaturated fatty acids (Fulco, 1983). Since, in addition to a high proportion of branched forms, the genus *Stenotrophomonas* had more monounsaturated acids compared to Gram positive bacteria, this genus seems to maintain optimal membrane fluidity at a given growth temperature. This feature also used to differentiate this genus readily from Gram negative genera examined, especially the genus *Pseudomonas* with trace amount of branched acids.

Unsaturated fatty acids were generally found in higher quantities in Gram negative bacteria than Gram positive (Oliver and Colwell, 1973). Moreover, it is well established that low temperature causes increase of unsaturated acids synthesis (Jones and Prahl, 1985). In fact, marine bacteria contain a higher degree of unsaturated rather than saturated fatty acid, since marine strains are mostly Gram negative bacteria and over 80% of the ocean is 5℃ or lower. Therefore, the result for Gram negative genera with a high contents of unsaturated acids of 23 to 63% of totals was consistant to that of the other investigations (Oliver and Colwell, 1973; Jones and Prahl, 1985). However, in Gram positive bacteria in which branched acids are prevalent, there is usually a corresponding decrease in the proportion of unsaturated forms. For example, it was reported that the proportion of unsaturated acids was low or nil in the genus *Staphylococcus* (O'Leary and Wilkinson, 1988), which is similar to that of this study.

Another interesting feature observed in unsaturated fatty acid profiles was that *cis*-monoenoic acids mainly detected, which are generally found in bacteria, in marine genus studied. This finding is consistent with results from other investigators. As pointed out by Gillan et al. (1981), *trans*-monounsaturated acids may not result from biological or chemical diagenetic processes. It has been demonstrated often that the *trans/cis* ratio of bacteria might change both quantitatively and qualitatively depending on the environmental conditions.

The survey of marine bacteria presented in Table 2 suggests that all of the studied genera possessed 16:0 straight fatty acid as one of the major constituents, and it accounted for 2 to 21% of totals and it was detected more in Gram negative than Gram positive genera. These features appeared to be similar to the reports of other investigators. Another notable feature of straight acids is the presence of 20:0, which reaches substantial level in the genus *Staphylococcus*, and this can be used as diagnostic component (O'Leary and Wilkinson, 1988).

Pseudomonads are a large group which, despite major advances in the phenotypic characterization of its members, remain as an uncomfortable diverse assemblage. This diversity extents to the lipid composition of these organisms and it follows, therefore, that fatty acids profiles have an important role to play in the identification and classification of species (Wilkinson, 1988). The most distinguishing feature of the genus *Pseudomonas* were the presence of cyclopropane fatty acid. It has been generally known that fatty acids containing a cyclopropane ring are commonly found in some of Gram negative bacteria but rarely in Gram positive species (Cho and Salton, 1966; Fulco, 1983). The basic mechanism for the formation of cyclopropanate in bacteria involves the methylation of a *cis*-monoenoic acids (Fulco, 1983). Furthermore, it have been found in higher concentrations when a longer incubation time or a higher growth temperature had been used (Padley et al., 1994).

Hydroxy (2- and 3-OH) fatty acids, in general, are predominantly found in Gram negative bacteria and most often linked to the lipopolysaccharides of the cell wall membrane (Tornabene, 1985). Kohring et al. (1994) suggested that the inclusion of hydroxy types in the analysis of fatty acid pattern might increase the precision of the analysis, and should be included to determine the phenotypic relationships based on similarities of fatty acid profiles.

The presence of one specific fatty acid may be a taxonomic marker for a species. When the index fatty acid species specific for certain bacteria, such as 10ME 16:0 for *Desulfobacter* (Kohring et al., 1994), were used, the microbial identification could be achieved with a high accuracy in the natural environments containing several bacterial species (Katayama-Fujimura et al., 1982; Taylor and Parkes, 1985; Tornabene, 1985; Lehtonen et al., 1996). Therefore, the community fatty acid profiles of

bacterial FAMEs can be used to assess the relative similarities and differences of microbial communities that differ in taxonomic composition (Haack et al., 1994). However, as pointed out by Wilkinson (1988), it is desirable that the fatty acids detected in rapid surveys should be characterized as fully and rigorously as possible.

It has been also suggested that the greater the knowledge concerning the fatty acid profiles of bacteria, the more readily will one be able to trace their presence in nature and estimate the relative biomasses and ecological contributions of various types of organisms (Taylor and Parkes, 1985; Lechevalier and Lechevalier, 1988). Thus, using the bacterial fatty acids, the structure and biomass of marine microbial communities can be effectively examined without the separation and identification process of cells.

References

Bertone, S., M. Giacomini, C. Ruggiero, C. Piccarolo and L. Calegari. 1996. Automated systems for identification of heterotrophic marine bacteria on the basis of their fatty acid composition. Appl. Environ. Microbiol., 62, 2122~2132.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911~917.

Cho, K.Y. and M.R.J. Salton. 1966. Fatty acid composition of bacterial membrane and wall lipids. Biochem. Biophys. Acta., 116, 73~79.

Fulco, A.J. 1983. Fatty acid metabolism in bacteria. Prog. Lipid Res., 22, 133~160.

Gillan, F.T., R.B. Johns, T.V. Verheyen, J.K. Volkman and H.J. Bavor. 1981. *trans*- Monounsaturated acids in a marine bacterial isolate. Appl. Environ. Microbiol., 41, 849~856.

Guckert, J.B., D.B. Ringelberg, D.C. White, R.S. Hanson and B.J. Bratina. 1991. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the proteobacteria. J. Gen. Microbiol., 137, 2631~2641.

Guckert, J.B., M.A. Hood and D.C. White. 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol., 52, 794~801.

Haack, S.K., H. Garchow, D.A. Odelson, L.J. Forney and M.J. Klug. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Appl. Environ. Microbiol., 60, 2483~2493.

Hanna, K., C. Bengis-Garber, D.J. Kushnerz, M. Kogut and M. Kates. 1984. The effect of salt concentration on the phospholipid and fatty acid composition of the moderate halophile *Vibrio costicola*. Can. J. Microbiol., 30, 669~675.

Jones, R.D. and F.G. Prahl. 1985. Lipid composition of a marine ammonium oxidizer grown at 5℃ and 25℃. Mar. Ecol. Prog. Ser., 26, 157~159.

Kaneda, T. 1991. Iso- and anteiso-fatty acids in bacteria: Biosynthesis, function, and taxonomic significance. Microbiol. Rev., 55, 288~302.

Kang, W.B., H.K. Seong, C.H. Moon and W.J. Lee. 1997. Distribution of marine bacteria and cellular fatty acid composition of dominated genus in Suyeong Bay. Bull. Kor. Fish. Soc., 30, 640~651.

Katayama-Fujimura, Y., N. Tsuzaki and H. Kuraishi. 1982. Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus*. J. Gen. Microbiol., 128, 1599~1611.

Keweloh, H. and H.J. Heipieper. 1996. *Trans* Unsaturated fatty acids in bacteria. Lipids, 31, 129~137.

Kieft, T.L., E. Wilch, K. O'Conner, D.B. Ringelberg and D.C. White. 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Appl. Environ. Microbiol., 63, 15 31~1542.

Kohring, L.L., D.B. Ringelberg, R. Devereux, D.A. Stahl, M.W. Mittelman and D.C. White. 1994. Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. FEMS Microbiol. Lett., 119, 303~308.

Lehtonen, L., R. Peltonen and E. Eerola. 1996. Computerized gas-liquid chromatography of bacterial cellular fatty acids in analysis of bacterial mixtures. J. Microbiol. Meth., 25, 317~327.

Lechevalier, H. and M.P. Lechevalier. 1988. Chemotaxonomic use of lipids-an overview. In *Microbial lipids*, Vol. 1, C. Ratledge and S.G. Wilkinson, eds. Academic Press, New York, pp. 869~902.

Miller, L.T. and T. Berger. 1985. Hewlett-Packard gas chromatography application note 228-41. Hewlett-Packard Co., Palo Alto, California.

Nichols, D.S., T.A. McMeekin and P.D. Nichols. 1994. Manipulation of polyunsaturated, branched-chain and *trans*-fatty acid production in *Shewanella putrefaciens* strain ACAM 342. Microbiology, 140, 577~584.

O'Leary, W.M. and S.G. Wilkinson. 1988. Gram-positive bacteria. In *Microbial lipids*, Vol. 1, C. Ratledge and S. G. Wilkinson, eds. Academic Press, New York, pp. 117~201.

Oliver, J.D. and R.R. Colwell. 1973. Extractable lipids of Gram-negative marine bacteria: Fatty-acid composition.

Int. J. System. Bacteriol., 23, 442~458.

Padley, F.B., F.D. Gunstone and J.L. Harwood. 1994. Occurrence and characteristics of oils and fats-Bacterial lipids. In *The lipid handbook*, F.D. Gunstone, J.L. Harwood and F.B. Padley, 2nd ed. Champman & Hall, London, pp. 216~222.

Seong, H.K., W.J. Lee, Y.H. Kim and K.J. Harm. 1992. Studies on bacterial characteristics of *Bacillus cereus* group LS-1 isolated from Suyeong Bay. Kor. J. Microbiol., 30, 339~346.

Shaw, N. 1974. Lipid composition as a guide to the classification of bacteria. Adv. Appl. Microbiol., 17, 63~108.

Taylor, J. and R.J. Parkes. 1985. Identifying different populaitons of sulphate reducing bacteria within marine sediment systems, using fatty acid biomarkers. J. Gen. Microbiol., 131, 631~642.

Tornabene, T.G. 1985. Lipid analysis and the relationship to chemotaxonomy. Meth. Microbiol., 18, 209~234.

Weber, F.J., S. Isken and J.A.M. de Bont. 1994. *cis/trans* Isomerization of fatty acids as a defence mechanism of *Pseudomonas putida* strains to toxic concentrations of toluene. Microbiology, 140, 2013~2017.

White, D.C., W.M. Davis, J.S. Nickels, J.D. King and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia, 40, 51~62.

Wilkinson, S.G. 1988. Gram-negative bacteria. In *Microbial lipids*, Vol. 1, C. Ratledge and S.G. Wilkinson, eds. Academic Press, New York, pp. 299~488.

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