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Fatty Acid Analysis to Determine the Seasonal Variation in Microbial Biomass and its Community Structure of Coastal Sediments

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Abstract In order to understand the seasonal variation in microbial biomass and its community structure in coastal sediments, phospholipid ester-linked fatty acids (PLFA) in sediments were analysed. The fatty acids consist of saturated, monounsaturated, branched and polyunsaturated fatty acids and most of them are reported to be characteristic fatty acids of microorganisms. The calculated microbial biomass showed marked seasonal variation during the study period with high microbial biomass in summer and low in winter. In spring, the calculated microbial biomass were higher than that observed in autumn and winter. Microbial community structure in the sediments, as determined by PLFA, was dominated by prokaryotes (high abundances of microbial biomarkers) with a relatively equal proportion of aerobic and anaerobic bacteria, and also eukaryotes. Among the anaerobic bacteria, the presence of sulfate reducing bacteria (SRB) was understood from the detection of signature fatty acids of SRB in the sediments. Relatively low amounts of microeukaryotic biomarker fatty acids (PUFA) in the sediments suggested that the distribution of microeukaryotes in the surface sediments was restricted because of the existing environmental condition. Microbial community structure in the sediments did not show significant seasonal variation during the study period.

Key words: Fatty acids, microbial biomass, community structure, sediments, pollution

INTRODUCTION

In Japan, eutrophication caused by nutrients has been regarded as a serious problem in many coastal bays. In shallow areas, increased algal biomass induces anoxia in the sediments resulting the release of nutrients, which again has positive feedback effect on algal blooms. Sediment provides habitat for many aquatic organisms but also a major repository for many pollutants and nutrients introduced into surface waters. Both the organic matter produced by the eutrophication and the pollutants introduced into the coastal bays have significantly affected the survival of aquatic life. Microorganisms in sediments are known to play a vital role in the decomposition of organic matter and degradation of organic pollutants in the sediments. Information about the microbial population in such an environment are imperative to understand the role played by the microorganisms (RAJENDRAN *et al.*, 1992c). However the estimation of microbial biomass and its community structure in sediments poses a problem to both microbiologists and environmental chemists.

Although a number of biochemical methods have been followed to determine the microbial biomass in sediments, all these methods tend to have certain limitations. Recently, special attention has been focused on the analysis of phospholipids in sediments for both qualitative and quantitative analyses of microorganisms in sediments. Fatty acid analysis has become a valuable tool for taxonomical and phylogenic classification (LECHEVALIER, 1977). The analysis of PLFA in sediments is presently one of the useful chemical methods to determine the microbial biomass (WHITE, 1983). Measurements of the chemical components of microbial cells, i.e., phospholipids can be used to identify viable members of microbial communities in sediments and to quantify cell biomass (BALKWILL *et al.*, 1988; WHITE, 1983, 1986; WHITE *et al.*, 1979). PLFA analysis allows us to assess the microbial biomass, community structure and metabolic status without problems associated with direct enumeration or culture methods (WHITE, 1986). Although the PLFA analysis has been extensively employed to compare the microbial community structure in sediments of different environments (BAIRD and WHITE, 1985; BAIRD *et al.*, 1985; BOBBIE and WHITE, 1980; RINGELBERG *et al.*, 1988; SMITH *et al.*, 1985; MANCUSO *et al.*, 1990; RAJENDRAN *et al.*, 1995b) or to understand the variation in microbial communities within the coastal environments in Japan (RAJENDRAN *et al.*, 1992a,b,c,d, 1993a,b, 1994) or to determine the difference between summer and winter seasons (RAJENDRAN *et al.*, 1995a), no attempt has been made to employ this method to understand the seasonal variation in microbial biomass and its community structure in sediments. Hence, in the present study, PLFA analysis was used to elucidate the seasonal variation of microbial biomass and its community structure in coastal sediments.

MATERIALS AND METHODS

Study area and sample collection

The present study area, Kure port, is located in the Hiroshima Bay which is reported to be affected by eutrophication (Fig. 1). The study area houses a ship building industry and is one of the biggest ports in the Seto Inland Sea of Japan. The present study area is contaminated by both industrial and domestic wastes. The Niko River is having its exit near the sampling station. Four sediment samples were collected during each season for one year from the selected station near the Kure Marine Station, Hiroshima University, Japan (34°14′N Lat., 132°33′E Long.) and the depth of the station is 10 m. The collected sediments were mainly consist of silty mud with hydrogen sulphide odor, and they are dark gray to black in color. The collected sediments were frozen at −20°C

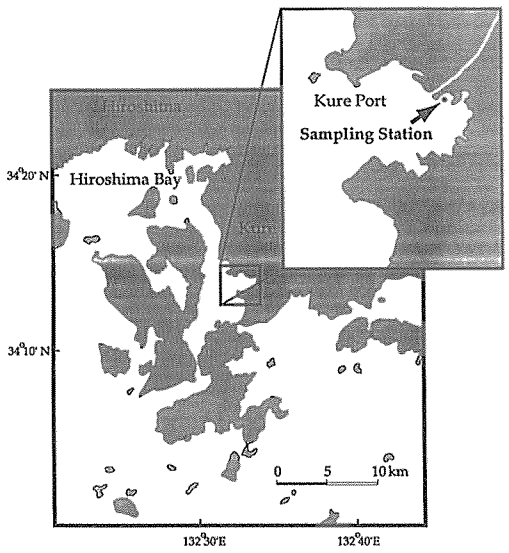

Fig. 1 Location of the sampling station.

until analysis.

Lipid extraction

Phosphate buffer, chloroform and methanol (2:3:6 v:v:v) were used to extract lipid from the freeze-dried sediment samples (BAIRD and WHITE, 1985). The extracted lipids were fractionated into neutral lipids, glycolipids, and phospholipids using silicic acid column chromatography. A mild alkaline methanolysis was performed to the phospholipid fraction of the lipids to release and methylate the ester-linked fatty acids (WHITE *et al.*, 1979).

Thin layer chromatography

The resultant fatty acid methyl esters were further purified by thin layer chromatography as described elsewhere (RAJENDRAN *et al.*, 1992c). The precoated silica gel plates (size 20 cm×20 cm×250 μm; E. Merck, Germany) were precleaned in hexane-diethyl ether (1:1 v:v) and then used for thin layer chromatography.

Gas chromatography (GC) and GC-mass spectrometry (GC-MS)

Gas chromatography analyses were carried out using a Hewlett Packard (HP 5890A) gas chromatograph equipped with a 25 m cross-linked 5% phenyl methylsilicone fused capillary column (0.2 mm i.d) and a flame ionization detector. Sample was injected by using HP 7673A automatic sampler in the splitless mode with a 30 sec venting time at 250°C. The temperature program of GC, as described earlier (RAJENDRAN *et al.*, 1992c), was followed. Helium was used as a carrier gas. Before GC-MS analysis, comparison of retention times with known standards of fatty acid methyl esters (Supelco Inc., U.S.A.) was made for tentative peak identification of compounds separated by GC. GC-MS analyses of PLFA samples were performed on a model HP 5890A gas chromatograph with a model HP 5970 series mass selective detector. The position and geometry of double bond in monounsaturated PLFA were determined by GC-MS analysis of the adducts following the DMDS reaction of the sample as described earlier (NICHOLS *et al.*, 1986). The nomenclature of PLFA followed in the present study has already been described (RAJENDRAN *et al.*, 1992c).

Statistical analysis

Tukey's Honestly Significant Difference (HSD) test was used to determine the significant difference among the means for each PLFA while maintaining an experiment-wise error rate of alpha=0.05. Tests were performed using a HITAC M-680H (VOS-3) program with a main frame computer available at the Hiroshima University Information Processing Saijo Center.

RESULTS

PLFA composition

The mean percentage and standard deviation of individual PLFA in the sediments collected during each season are shown in Table 1. Sixty-three PLFA were identified in the sediments and they were in the range of C10-25. They consist of saturated fatty acids, branched fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (Table 2). High percentages of 16:0 fatty acid were determined in all the samples. The fatty acids in the range of C12-19 are reported to be bacterial origin (LECHEVALIER, 1977). In the range of C12-19, even numbered saturated fatty acids were present in higher percentages than branched and unsaturated fatty acids (Table 2), whereas, in autumn samples, the

Table 1. Percent composition of PLFA in coastal sediments*

Fatty acid	Summer	Autumn	Winter	Spring
10:0	0.05±0.06	0.84±0.67	0.01±0.04	0.07±0.05
i12:0	0.20±0.14	0.24±0.20	0.07±0.17	0.19±0.10
a12:0	0.29±0.39	—	—	0.12±0.09
12:0	0.69±0.50	0.55±0.28	0.96±0.36	0.73±0.28
i13:0	0.22±0.25	0.12±0.08	0.32±0.22	0.19±0.13
a13:0	0.90±0.60	1.22±0.16	0.74±1.06	0.99±0.35
13:0	0.32±0.26	0.09±0.07	0.18±0.21	0.12±0.11
br14:1	0.64±0.46	0.89±0.58	1.24±1.57	1.87±0.42
i14:0	1.51±0.61	1.31±0.36	2.10±0.40	1.85±0.30
a14:0	0.67±0.77	1.26±0.81	0.60±0.70	0.86±0.44
14:1d7	—	0.42±0.28	0.72±0.35	0.60±0.54
14:1d9	0.31±0.36	0.56±0.21	0.64±0.18	0.72±0.23
14:2	0.96±1.10	1.40±1.23	0.32±0.64	0.72±0.47
14:0	6.62±1.38	4.26±0.89	8.63±1.33	7.72±0.78
i15:1	0.69±0.46	0.44±0.22	0.11±0.13	0.44±0.30
a15:1	0.32±0.37	0.35±0.24	0.21±0.14	0.67±0.13
i15:0	6.59±1.34	4.70±1.01	6.76±1.32	6.02±0.88
a15:0	8.22±2.32	7.52±2.97	8.89±1.96	7.64±1.71
15:1d7	0.25±0.29	—	—	0.20±0.14
15:1d9	0.14±0.16	0.08±0.10	0.12±0.25	0.19±0.08
15:0	3.04±1.55	1.76±0.27	2.42±0.54	2.06±0.03
16:2	0.15±0.17	0.17±0.19	0.18±0.21	0.04±0.08
i16:0	1.93±0.30	1.90±0.25	2.23±0.26	1.86±0.24
16:1d6	0.33±0.38	0.49±0.10	0.15±0.17	0.48±0.33
16:1d7	0.74±0.55	0.92±0.64	0.70±0.48	0.80±0.50
16:1d9*c*	9.23±0.64	8.60±1.16	8.43±1.42	8.21±2.03
16:1d9*t*	1.45±0.16	1.02±0.32	1.36±0.45	1.31±0.30
16:1d11*c*	1.74±0.05	1.67±0.28	1.76±0.38	1.52±0.29
16:1d11*t*	0.15±0.18	0.14±0.16	—	0.33±0.23
16:0	19.63±2.67	16.81±4.65	20.24±0.14	19.54±0.96
br17:1	—	0.24±0.18	—	0.11±0.07
10Me16:0	1.78±0.97	3.03±1.12	1.72±1.01	2.57±0.84
i17:1	0.32±0.22	0.33±0.23	0.21±0.24	0.26±0.18
a17:1	1.00±1.00	0.80±0.17	0.74±0.50	0.42±0.41
i17:0	0.97±0.19	1.02±0.13	1.08±0.05	0.92±0.05
a17:0	1.41±0.60	1.73±0.36	1.80±0.10	1.57±0.28
17:1d9*c*	0.51±0.35	0.68±0.20	0.51±0.34	0.63±0.04
17:1d9*t*	0.75±0.52	0.59±0.44	0.40±0.40	0.84±0.12
cy17:0	0.07±0.08	—	—	—
17:0	1.24±0.16	1.29±0.14	1.30±0.03	1.23±0.13
i18:0	0.04±0.05	—	—	0.19±0.18
a18:0	0.51±0.80	0.15±0.17	—	0.25±0.17
18:2	1.01±0.56	1.23±0.33	1.42±0.40	1.09±0.39
18:1d9*c*	2.11±1.80	1.22±0.24	1.28±0.22	1.17±0.04
18:1d9*t*	4.79±1.23	5.54±2.19	4.26±0.13	3.93±0.71
18:1d11*c*	4.21±2.86	6.73±1.58	5.35±0.88	5.00±0.67

*Mean and standard deviation

Table 1. contd.

Fatty acid	Summer	Autumn	Winter	Spring
18:1d11*t*	0.38±0.26	0.29±0.21	0.43±0.30	0.47±0.04
18:1	0.08±0.09	0.11±0.12	—	0.08±0.09
18:0	3.99±1.22	4.29±1.61	3.33±0.52	3.15±0.61
br19:1	0.88±0.88	0.66±0.14	0.66±0.24	0.43±0.33
10Me18:0	0.07±0.08	0.30±0.20	0.03±0.06	0.04±0.07
cy19:0	0.08±0.10	0.23±0.16	0.13±0.15	0.16±0.11
20:5	0.58±0.15	1.75±1.75	0.32±0.29	0.35±0.25
20:4	1.69±0.93	2.72±2.36	0.78±0.27	0.93±0.69
20:1d11*c*	0.70±0.79	1.65±1.65	0.62±0.71	0.48±0.04
20:1d11*t*	0.16±0.18	0.06±0.12	—	0.19±0.13
20:1	0.23±0.27	0.17±0.14	0.23±0.45	0.43±0.40
20:0	0.34±0.26	0.54±0.20	0.62±0.15	0.49±0.15
22:1	0.68±1.14	0.45±0.52	—	0.19±0.14
22:0	0.23±0.23	0.31±0.19	0.16±0.18	0.32±0.19
24:0	0.63±0.82	0.11±0.08	0.10±0.12	0.28±0.08
25:0	0.61±1.22	2.05±1.75	2.42±4.29	3.76±3.17

*Mean and standard deviation

Table 2. Seasonal variation of different groups of PLFA (%) in sediments of the study area*

PLFA group	Summer	Autumn	Winter	Spring
Even numbered saturated PLFA (<19)	31.0±2.0	26.8±5.9	33.2±1.3	31.2±1.1
Odd numbered saturated PLFA (<20)	4.6±1.7	3.1±0.2	3.9±0.7	3.4±0.1
Branched PLFA	29.0±3.5	28.1±6.0	29.4±1.2	29.0±2.8
Monounsaturated PLFA (<19)	27.5±3.7	29.4±2.7	26.3±3.8	27.2±3.0
Polyunsaturated PLFA	2.1±0.8	2.8±1.5	1.9±0.3	1.8±0.7
Saturated PLFA (>20)	1.8±1.6	3.0±1.5	3.3±4.2	4.8±3.5
Monounsaturated PLFA (>20)	4.0±2.8	6.8±6.3	2.0±1.2	2.6±0.9

*Mean and standard deviation

even numbered saturated fatty acids were detected in lower percentages than branched and monounsaturated fatty acids. Low percentages of odd numbered saturated fatty acids were present in all the seasons. Monounsaturated fatty acids longer than 20 carbon atoms and polyunsaturated fatty acids were also present in small amounts. Significant amounts of saturated fatty acids 14:0, 16:0, and 18:0 were present. Branched fatty acids iso and anteiso PLFA in the range of C12-18 were detected. Methyl branching fatty acids 16:0 and 18:0 were also present in the sediments. Low amounts of cyclopropyl fatty acids of C17 and 19 detected in the sediments. Monounsaturated fatty acids with chain length shorter than 20 were in the range of C14-18 and the amounts of *cis* isomer of 16:1d9 were

Table 3. Seasonal variation in calculated microbial biomass and other ratios in sediments*

Parameter	Summer	Autumn	Winter	Spring
Microbial biomass (10^7 cells/g)	0.77±0.48	0.56±0.23	0.49±0.22	0.69±0.35
Monounsaturated fatty acids<19/branched fatty acids	0.96±0.17	1.08±0.27	0.90±0.14	0.94±0.15
16:1d9*t*/*c*	0.16±0.01	0.12±0.04	0.16±0.03	0.16±0.00
i±a 15:0/16:0	0.77±0.24	0.75±0.28	0.77±0.15	0.70±0.16

*Mean and standard deviation

higher than the *trans* isomer (Table 1). In general, the number of fatty acids present in the sediments did not show much variation among the seasons.

Microbial biomass

The mean total PLFA concentration in sediment showed marked variation among the seasons, ranging from 1.12 (winter) to 1.78 (summer) μg/g dry weight sediment (Fig. 2). During the study period, the highest amount of total PLFA was observed in summer and then it reached to the lowest value in winter, and in spring, the PLFA concentration showed an increasing trend. The microbial biomass in sediments were calculated by following the factors reported by BALKWILL *et al.* (1988); WHITE *et al.* (1979) and MANCUSO *et al.* (1990). Microbial biomass thus calculated using these conversion factor ranged from 4.9×10^7 (winter) to 7.7×10^7 (summer) cells/g dry weight (Fig. 2). The calculated biomass were abundantly present in summer and then the biomass showed a decreasing trend to reach the lowest biomass in winter. In spring, the biomass was higher than that observed in winter and autumn (Fig. 2).

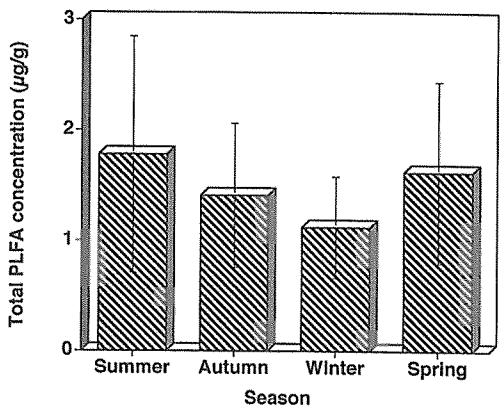

Fig. 2 Seasonal variation in total PLFA concentration of sediments.

Since the branched and monounsaturated fatty acids are reported to be marker fatty acids for anaerobic and aerobic bacteria respectively, the ratios of these fatty acids will provide relative dominance of these microbial groups (RAJENDRAN *et al.*, 1992c). During the study period, these ratios ranged from 0.90 (winter) to 1.08 (Autumn) indicate that these two groups of bacteria did not show much variation (Table 3). Similarly the ratios iso and anteiso 15:0 to 16:0 will provide the relative distribution of bacterial biomarker fatty acids (MANCUSO *et al.*, 1990) and these ratios also did not show any variation during the study period (Table 3), revealing not much difference in the lipid contributing microbial communities during the study period. In addition to the microbial biomass and community structure in sediments, the PLFA composition are also reported to provide information about the nutritional status of the microorganisms (GUCKERT *et al.*, 1985, 1986). In bacteria

Table 4. Significant difference in mean values of PLFA in sediments collected during the four seasons*

Fatty acid	Low			High
10:0	Winter	Summer	Spring	Autumn
i14:0	Autumn	Summer	Spring	Winter
14:0	Autumn	Summer	Spring	Winter
i15:1	Winter	Autumn	Spring	Summer
a15:1	Winter	Summer	Autumn	Spring
10Me18:0	Winter	Spring	Summer	Autumn

*Values increase from left to right and the values connected by a line are not significantly different.

and sediments, the ratio of *trans* and *cis* of 16:1d9 are reported to be 0.1 (GILLAN and HOGG, 1984; GUCKERT *et al.*, 1985, 1986; PERRY *et al.*, 1979) but they are greater than 1 during starvation. During the study period, the ratios of *t*/*c* of 16:1d9 were in the range of 0.12 to 0.16 (Table 3), indicating that the organisms might have been exposed to some physiological stress.

Microbial community structure

Microbial community structure in sediments of Kure port showed the greater dominance of prokaryotes, as characterized from the high proportions of microbial marker fatty acids in the range of C12-19 and relatively a very low presence of microeukaryotes, as evidenced by the low amounts of microeukaryotic biomarker fatty acids (less than 3% of polyunsaturated fatty acids). The means of each PLFA that are significantly different among the seasons are shown in Table 4. Except six fatty acids, a majority of PLFA in sediments did not show any significant difference among the four seasons, indicating the absence of variation in microbial community structure during the study period. The relative dominance of microbial groups in sediments can be explained using the relative proportions of reported biomarker fatty acids (FINDLAY *et al.*, 1990, 1993). The employment of this classification revealed the presence of microeukaryotes, aerobic prokaryotes and eukaryotes, anaerobic bacteria and SRB in the present study area (Fig. 3).

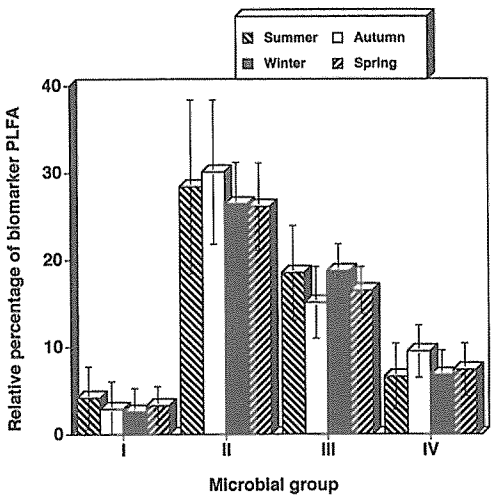

Fig. 3 Seasonal variation in the relative proportion of microbial groups in sediments (I: Microeukaryotes; II: aerobic prokaryotes and eukaryotes; III: anaerobic bacteria; IV: SRB and other anaerobic prokaryotes).

DISCUSSION

PLFA analysis has been used to determine the microbial biomass and community structure in estuarine and deep-sea (BAIRD and WHITE, 1985; BAIRD *et al.*, 1985), coastal (RAJENDRAN *et al.*, 1992a,b,c,d,

1993a,b, 1994, 1995a,b), Antarctic lake (MANCUSO *et al.*, 1990) and polluted sediments (SMITH *et al.*, 1985). Of the sixty-three fatty acids identified in the sediment, most of the PLFA are reported to be microbial in origin. Fatty acids in the range of C12-19 are reported to be characteristic of bacteria (LECHEVALIER, 1977). Similar patterns of PLFA profiles were reported in sediments of Hiroshima Bay (RAJENDRAN *et al.*, 1992c,d), Osaka Bay (RAJENDRAN *et al.*, 1992a, 1994), and Kojima Lake (RAJENDRAN *et al.*, 1995a). The distribution of branched, monounsaturated, cyclopropyl and certain saturated fatty acids in sediments has been attributed to the bacterial contribution (GILLAN and HOGG, 1984; PERRY *et al.*, 1979; VOLKMAN *et al.*, 1980). High percentages of 16:0 (17 to 20% of total PLFA), which is commonly present in most organisms, were determined in sediments (Table 1). Branched fatty acids are reported as signature fatty acids of bacteria (VOLKMAN *et al.*, 1980; WHITE, 1983), anaerobic bacteria (KANEDA, 1977; TAYLOR and PARKER, 1983), and SRB (BOON *et al.*, 1977, 1978; EDLUND *et al.*, 1985). The characteristic fatty acids, iso and anteiso of 15:1 and 17:1, of SRB *Desulfovibrio* spp (EDLUND *et al.*, 1985) were detected in the sediment samples of Kure. The fatty acid profile of SRB *Desulfobacter* spp was dominated by 10Me16:0 that was not detected in any other SRB and this fatty acid has been proposed as signature fatty acid of *Desulfobactor* spp (EDLUND *et al.*, 1985). In the pesent study period, the detection of these branched PLFA 10Me16:0, iso and anteiso 15:1 and 17:1 in sediments samples indicated the presence of these SRB. The predominance of these branched fatty acids in the PLFA profiles make them useful bacterial indicator (PERRY *et al.*, 1979) and their greater proportion (28 to 29% of total PLFA) in the present study area must be contributed by anaerobic bacteria and of SRB. The distribution of SRB in sediments of the present study area could be further confirmed by the reported sulphide concentration (0.07 to 0.24 mgS/g dry weight) in sediments (IMAMURA, 1991) and the seasonal variation in the distribution of SRB in sediments of Hiroshima Bay (OKADA, 1991). Like branched fatty acids, monounsaturated fatty acids in sediments are indicative of bacterial contribution (GILLAN *et al.*, 1983; PARKES and TAYLOR, 1983; PERRY *et al.*, 1979). High amounts of monounsaturated fatty acids (26 to 29% of total PLFA) in sediments of the present study area are certainly contributed by the bacteria in sediments.

Polyunsaturated fatty acids in sediments are indicative of microeukaryotic input (MANCUSO *et al.*, 1990; VOLKMAN *et al.*, 1980). The mean percentage of polyunsaturated fatty acids in sediments ranged from 1.8 to 2.8% of the total PLFA (Table 2). Considerably low amounts polyunsaturated fatty acids, which are characteristics of eukaryotes, in the PLFA profiles of the sediments strongly suggested that the PLFA are definitely derived from bacteria in the sediments. Hydrographical investigation carried out during the study period showed that the concentrations of dissolved oxygen in the bottom water samples (2 m above the sediment) were low (NAKANISHI, 1990) and was expected to be lower at the sediment-water interface. During summer and autumn, the depletion of oxygen in the bottom waters of Hiroshima Bay and the absence of detectable oxidized sediment layer because of lack of vertical mixing have been reported (RAJENDRAN *et al.*, 1992c,d; IMAMURA, 1991). Hiroshima Bay is reported to be highly polluted because of artificial eutrophication. As reported by FINDLAY *et al.* (1990), the reduced availability of oxygen at the sediment-water interface probably reduced the survival of microeukryotes in the surface sediments.

Relatively low amounts or absence of polyunsaturated fatty acids in sediments of eutrophic bays have been attributed to the reduced condition of the sediment, the reduced availability of dissolved oxygen, and presence of sulfide in the bottom waters and the organic pollution (RAJENDRAN *et al.*, 1992c). In addition to the eutrophication in the Kure port which is a part of Hiroshima Bay, the port is reported to be polluted by both domestic and industrial wastes. The survival of microeukaryotes in sediments of many Japanese coastal bays, including Hiroshima Bay are found to be affected by the organic pollution and eutrophication. The results of SMITH *et al.* (1985) and RAJENDRAN *et al.* (1992b, 1993b) also supports the absence of microeukaryotic contribution of fatty acids to sediments of the present study area.

The calculated microbial biomass showed marked seasonal variation during the study period (Table 3). The calculated microbial biomass agreed with the calculated biomass in sediments of Hiroshima Bay and its adjacent bays (RAJENDRAN *et al.*, 1992c,d), Kojima Lake (RAJENDRAN *et al.*, 1995a) and Osaka Bay (RAJENDRAN *et al.* 1992a, 1994). Seasonal variation in microbial biomass could be attributed to the environmental characteristics of the study area. The oceanographical characteristics of the present study area showed marked seasonal variation with low temperature and chlorophyll *a* in winter than other seasons and not much variation in salinity and density of the water samples (MATSUDA *et al.*, 1990). Furthermore, seasonal variation of bacteria in the bottom water samples (2 m above the sediment) determined by the acridine orange direct counts (NAKANISHI, 1990) was similar to the seasonal variation observed in the present investigation. Similarly, polarlipid fatty acid analysis of anoxic sediments of Kojima Lake showed that the calculated microbial biomass were higher in summer than that in winter (RAJENDRAN *et al.*, 1995a). Microbiological investigations carried out in the Seto Inland Sea of Japan (VENKATESWARAN *et al.*, 1989a,b) also showed low microbial biomass in winter than that observed in other seasons, and they pointed out that temperature was reported to exert a major influence on the microbial biomass.

The PLFA profiles of sediments dominated by a increased proportions of microbial biomarkers revealed that the microbial contribution of lipid to the sedimentary lipid is the major one. The distribution of the microeukaryotes, as evidenced by very low eukaryotic biomarkers because of the environmental conditions of the study area, are rather very much limited. The observed microbial community structure in sediments is similar to the reported microbial community structure in sediments of Hiroshima Bay (RAJENDRAN *et al.*, 1992c,d; 1993a), Kojima Lake (RAJENDRAN *et al.*, 1995a), Ise Bay (RAJENDRAN *et al.*, 1992b, 1993b) and Osaka Bay (RAJENDRAN *et al.*, 1992a, 1994). The results of Tukey's HSD test showed that most of the PLFA, except six fatty acids (Table 4), did not show any significant difference among the seasons. The absence of significant difference among the means of each PLFA suggests that the microbial community structure was not significantly different among the seasons. It could be further confirmed from the lack of variation in the iso and anteiso of 15:0 to 16:0 ratio (Table 3) during the study period.

From the results of the present investigation, it can be concluded that sedimentary microbial community structure was dominated by aerobic prokaryotes and eukaryotes, followed by anaerobic bacteria, SRB and microeukaryotes. The lack of significant dif-

ference in PLFA profiles indicated the absence of seasonal variation in microbial community structure. Since the number of sample is limited to four in the present study, it may be difficult to confirm the absence of seasonal variation. Hence it has been planned to collect a large number of samples during each season to know the seasonal variation in microbial community structure. However, marked seasonal variation in microbial biomass is understood.

REFERENCES

BAIRD, B. H. and WHITE, D. C. 1985. Biomass and community structure of the abyssal microbiota determined from the ester-linked phospholipids recovered from Venezuela basin and Puerto Rico Trench sediments. *Mar. Geol.*, 68:217-231.

BAIRD, B. H., NIVENS, D. E., PARKER, J. H. and WHITE, D. C. 1985. The biomass and community structure and spatial distribution of the sedimentary microbiota from high energy area of the deep sea. *Deep-sea Res.*, 32:1089-1099.

BALKWILL, D. L., LEACH, F. R., WILSON, J. T., MCNABB, J. F. and WHITE, D. C. 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate and direct counts in subsurface aquifer sediment. *Microb. Ecol.*, 16:73-84.

BOBBIE, R. J. and WHITE, D. C. 1980. Characterization of benthic microbial community structure by high resolution gas chromatography of fatty acid methyl esters. *Appl. Environ. Microbiol.*, 39: 1212-1222.

BOON, J. J., DE LEEUW, J. W., VAN DER HOEK, G. J. and VOSJAN, J. H. 1977. Significance and taxonomic value of iso and anteiso monoenoic fatty acids and branched ß-hydroxy acids in *Desulfovibrio desulfuricans*. *J. Bacteriol.* 129:1183-1191.

BOON, J. J., LIEFKENS, W., RIJPSTRA, W. I. C., BAAS, M. and DE LEEUW, J. W. 1978. Fatty acids of *Desulfovibrio desufuricans* as marker molecules in sedimentary environments, In Environmental and Geomicrobiology Biogeochemistry (KRUMBEIN, W. E., Ed.), Ann Arbor Science Publishers, Ann Arbor, Mich, pp. 355-372.

EDLUND, A., NICHOLS, P. D., ROFFEY, R., and WHITE, D. C. 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* speceis. *J. Lipid Res.*, 26:982-988.

FINDLAY, R. H., TREXLER, M. B., GUCKERT, J. B. and WHITE, D. C. 1990. Laboratory study of disturbance in marine sediments: response of a microbial community. *Mar. Ecol. Prog. Ser.*, 62:121-133.

FINDLAY, R. H., and DOBBS, F. C. 1993. Quantitative description of microbial communities using lipid analysis. In: Handbook of methods in Aquatic Microbial Ecology (KEMP, P. F., SHERR, B. F., SHERR, E. B., and COLE, J. J., ed), Lewis publishers, pp. 271-284.

GILLAN, F. T., JOHNS, R. B., VERHEY, Tk. V. and Nichols, P. D. 1983. Monounsaturated fatty acids as specific bacterial markers in marine sediments, In Advances in Organic Geochemistry (BJOROY, M., Ed), John Wiley and Sons Inc., New York, pp 198-206.

GILLAN, F. T. and HOGG, R. W. 1984. A method for the estimation of bacterial biomass and community structure in mangrove associated sediments. *J. Microbiol. Meth.*, 2:275-293.

GUCKERT, J. B., ANTWORTH, C. P., NICHOLS, P. D. and WHITE, D. C. 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol. Ecol.*, 31:147-158.

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

KANEDA, T. 1977. Fatty acids of the genus *Bacillus*: an example of branched chain preference. *Bacteriol. Rev.*, 41:391-418.

IMAMURA, N. 1991. Sulfide production and reduction in relation to the oxidation-redcution potential of sediments in eutrophic coastal regions. M. S. thesis, Hiroshima University, Hiroshima, Japan (In Japanese).

LECHEVALIER, M. P. 1977. Lipids in bacterial taxonomy a taxonomist's view. *Crit. Rev. Microbiol.*, 5: 109-210.

MANCUSO, C. A., FRANZMANN, P. D., BURTON, H. R. and NICHOLS, P. D. 1990. Microbial community structure and biomass estimates of a methogenic Antarctic Lake ecosystem as determined by phospholipid analyses. *Microb. Ecol.*, 19:73-95.

MATSUDA, O., KISHIMOTO, Y. and HAMASAKI, S. 1990. Factors affecting seasonal variation of oceanographic conditions at Kure port. *J. Fac. Appl. Biol. Sci. Hiroshima Univ.*, 29:135-144.

NAKANISHI, K. 1990. Factors affecting the downward flux of particulate organic matter in the Seto Inland Sea of Japan. M. S. thesis, Hiroshima University, Hiroshima, Japan (In Japanese).

NICHOLS, P. D., GUCKERT, J. B. and WHITE, D. C. 1986. Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. *J. Microbiol. Meth.*, 5:49-55.

OKADA, T. 1991. Study on the seasonal variations of sediments characteristics and sulphate reducing bacteria in eutrophic bays. M. S. thesis, Hiroshima University, Hiroshima, Japan (In Japanese).

PARKES, R. J. and TAYLOR, J. 1983. The relationship between fatty acid distribution and bacterial respiratory types in contemporary marine sediments. *Estuarine, Coastal and Shelf Sci.*, 16:173-189.

PERRY, G. J., VOLKMAN, J. K. and JOHNS, R. B. 1979. Fatty acids of bacterial origin in contemporary marine sediments. *Geochim Cosmochim Acta.*, 43:1715-1725.

RAJENDRAN, N., MATSUDA, O. and URUSHIGAWA, Y. 1992a. Distribution of polarlipid fatty acid biomarkers for bacteria in sediments of a polluted bay. *Microbios*, 72:143-152.

RAJENDRAN, N., SUWA, Y. and URUSHIGAWA, Y. 1992b. Microbial community structure in sediments of a polluted bay as determined by phospholipid ester-linked fatty acids. *Mar. Pollut. Bull.*, 24:305-309.

RAJENDRAN, N., MATSUDA, O., IMAMURA, N. and URUSHIGAWA, Y. 1992c. Variation in microbial biomass and community structure in sediments of eutrophic bays as determined by phospholipid ester-linked fatty acids. *Appl. Environ. Microbiol.*, 58:562-571.

RAJENDRAN, N., MATSUDA, O. IMAMURA, N. and URUSHIGAWA, Y. 1992d. Determination of microbial biomass and its community structure from the distribution of phospholipid ester-linked fatty acids in sediments of Hiroshima Bay and its adjacent bays. *Estuarine, Coastal and Shelf Sci.*, 36:501-514.

RAJENDRAN, N., MATSUDA, O. and URUSHIGAWA, Y. 1993a. Microbial community structure in the sediments of Hiroshima Bay. *Microbios*, 75:205-215.

RAJENDRAN, N., SUWA, Y. and URUSHIGAWA, Y. 1993b. Distribution of phospholipid ester-linked fatty acid biomarkers for bacteria in the sediments of Ise Bay. *Mar. Chem.*, 42:39-56.

RAJENDRAN, N., MATSUDA, O., URUSHIGAWA, Y. and SIMIDU, U. 1994. Characterization of microbial community structure in the surface sediment of Osaka Bay, Japan, by phospholipid fatty acid analysis. *Appl. Environ. Microbiol.*, 60:248-257.

RAJENDRAN, N., MATSUDA, O., IMAMURA, N. and URUSHIGAWA, Y. 1995a. Microbial community structure analysis of euxinic sediments using phospholipid fatty acid biomarkers. *J. Oceanogr.*, 51:21-38.

RAJENDRAN, N., MATSUDA, O. and SAKOH, H. 1995b. Comparison of microbial community structure in surface sediments of Saroma Ko lagoon with Seto Inland Sea in winter. *Proc. NIPR Symp. Polar Biol.*, 8:37-47.

RINGELBERG, D. B., DAVIS, J. D., SMITH, G. A., PFIFFNER, S. M., NICHOLS, P. D., NICKELS, J. S., HENSON,

J. M., WILSON, J. T., YATES, M., KAMPBELL, D. H., READ, H. W., STOCKSDALE, T. T. and WHITE, D. C. 1988. Validation of signature phospholipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. *FEMS Microbiol. Ecol.*, 62:39-50.

SMITH, G. A., NICKELS, J. S. KERGER, B. D., DAVIS, J. D., COLLINS, S. P., WILSON, J. T., MCNABB, J. F. and WHITE, D. C. 1985. Quantitative characterization of microbial biomass and community structure in surface material: a prokaryotic consortium responsive to organic contamination. *Can. J. Microbiol.*, 32:104-111.

TAYLOR, J. and PARKES, R. J. 1983. The cellular fatty acids of the sulphate reducing bacteria, *Desulfobacter* sp., *Desulfobulbus* sp., and *Desulfovibrio desulfuricans*. *J. Gen. Microbiol.*, 129: 3303-3309.

VENKATESWARAN, K., KIM, S. W., NAKANO, H., ONBE, T. and HASHIMOTO, H. 1989. The association of *Vibrio parahaemolyticus* serotypes with zooplankton and its relationship with bacterial indicators of pollution. *Syst. Appl. Microbiol.*, 11:194-201.

VENKATESWARAN, K., NAKANO, H., OKABE, T., TAKAYAMA, K., MATSUDA, O. and HASHIMOTO, H. 1989. Occurrence and distribution of *Vibrio* spp., *Listonella* spp. and *Clostridium botulinum* in the Seto Inland Sea, Japan. *Appl. Environ. Microbiol.*, 55:559-567.

VOLKMAN, J. K., JOHNS, R. B., GILLAN, F. T., PERRY, G. J. and BAVOR, Jr., H. J. 1980. Microbial lipids of an intertidal sediment I. Fatty acids and hydrocarbons. *Geochim Cosmochim Acta.*, 44: 1133-1143.

WHITE, D. C. 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. *Soc. Gen. Microbiol. Sym.*, 34:37-66.

WHITE, D. C. 1986. Quantitative physiochemical characterization of bacterial habitats, In Bacteria in nature, vol. 2 (POINDEXTER, J. S., and LEADBETTER, E. R., Ed.), Plenum Press, New York, pp. 177-203.

WHITE, D. C., DAVIS, W. M., NICKELS, J. S., KING, J. D. and BOBBIE, R. J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oceologia*, 40:51-62.

リン脂質の分析による沿岸堆積物中微生物バイオマスと群集構造の季節変動解析

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沿岸堆積物中における微生物のバイオマスと群集構造の季節変動を明らかにするために，バイオマーカー脂肪酸 (Phospholipid ester-linked fatty acids; PLFA) の分析を行った。これらは飽和脂肪酸，分枝脂肪酸，モノ不飽和脂肪酸あるいはポリ不飽和脂肪酸からなり，それらの大部分はそこに存在する微生物群機能群に特有な脂肪酸であった。微生物バイオマスの計算結果によれば，細菌のバイオマスは夏季に最大となり冬季に最小となる顕著な季節変動を示した。このバイオマスは春季には秋季や冬季よりも大きくなった。PFLAの分析結果から，堆積物中の微生物群集構造の特徴として，多量の細菌バイオマーカーの存在から分かるように原核生物が優占することと好気性細菌，嫌気性細菌および真核生物が比較的均等に分布することが明らかとなった。嫌気性細菌の中には，硫酸塩還元細菌に特有な指標性脂質の存在から，硫酸塩還元細菌が存在することも理解された。真核微生物の指標物質であるポリ不飽和脂肪酸 (PUFA) が概して堆積物中に極めて少ないことは真核微生物の生育がその場の堆積物環境に制限されていることを示唆するものである。研究期間中に微生物群集構造の顕著な季節的変動は観測されなかった。

キーワード：脂肪酸，微生物バイオマス，群集構造，堆積物，汚染