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# Microbial Biomarker Fatty Acid Composition in Coastal Sediments

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**Abstract** In the present study, the distribution of phospholipid ester-linked fatty acid composition (PLFA) in sediments were analyzed to determine the microbial biomass and microbial community structure. Thirty three fatty acids were identified in sediment samples analyzed for PLFA composition. Many fatty acids commonly considered to be indicative of bacterial contribution to sediments were identified in the study area. The calculated microbial biomass ranged from $1.5\times10^7$ to $9.3\times10^7$ cells/g dry weight of the sediment. Similarity analysis of the fatty acid composition revealed four different clusters and the possible microbial community structure is understood. The aerobic and anaerobic bacteria especially sulphate reducing bacteria are commonly distributed in the coastal sediment as evidenced by the relative proportion of unsaturated fatty acids and branched fatty acids respectively in total PLFA of sediments which are characteristic fatty acids of these bacteria.

## INTRODUCTION

The determination of microbial community and population *in situ* is one of the most difficult problems in microbial ecology, because of the difficulties involved in isolation and count of all microbes present in the environment without distortion of the *in situ* population. These difficulties are compounded by the fact that classical methods for recovering bacteria from natural habitats are highly inefficient and depending on the ecosystem sampled, capable of recovering 1% or less of the total cell numbers as judged using direct counts (BROCK, 1987). As pointed out by KING and WHITE (1977), classical plate counts yield considerably lower numbers than direct counts or biochemical measures of microbial biomass in sediments. In addition to the problems of providing a universal growth medium in the petriplate, the organisms must be removed from the sediment surfaces as well as from each other. The epifluorescent microscope method involves various disruptive techniques for the removal of microbes from sediments or biofilms (DALE, 1974) and the stripping is also supposed to be selective and often not quantitative (WHITE *et al.*, 1979). The stain, staining conditions and the exciting ultraviolet sources may alter the yield from a single suspension (DALEY and HOBBIE, 1975). The application of computer based image enhancing

can allow calculations of microbial biomass in complex assemblies (CALDWELL and GERMIDA, 1984). However, this methodology provides satisfactory results only when the density of organisms in sediments or biofilms is low and overlapping is minimal (WHITE, 1988).

A new technique using microbial biomarker has recently been used in many environments (PARKES, 1987). Microbial biomarkers are chemical components specific to microorganisms which can be analyzed directly from environmental samples and thus provide an alternative to enrichment techniques for the *in situ* study of indigenous microbial populations and can also be used to describe and quantify microbial biomass and community structure (WHITE, 1983). The lipid composition of aquatic sediments reflect the input of sedimented organic matter that has been exposed to biological alteration in addition to the direct contribution by organisms in sediments. Cell membrane lipid and its associated fatty acids as biomarkers have been used by many researchers as they are essential components of every living cell and have great structural diversity coupled with high biological specificity (PARKES, 1987). Such properties enable fatty acids to be used to study the complex sedimentary communities *in situ* thus avoiding the limitations of isolation techniques (GILLAN *et al.*, 1983; WHITE, 1983).

In this paper, phospholipid ester-linked fatty acid profiles in sediments of different environments are reported with special reference to microbial biomass and community structure. Phospholipids can be used to identify viable members of microbial communities in nature and to quantify cell biomass (BAIRD and WHITE, 1985; BALKWILL *et al.*, 1988; RINGELBERG *et al.*, 1988; WHITE, 1983 and 1986; WHITE *et al.*, 1979). WHITE *et al.* (1979) reported that the bacteria in sediments contain a relatively constant proportion of their biomass as phospholipids. Phospholipid ester-linked fatty acids (PLFA) are particularly useful biomarkers since they are essential components of every living cells which are discontinuously distributed amongst microbial taxa and readily extracted from environmental samples. In many studies, they are regarded as being unique to particular organisms and are therefore used to indicate the presence and relative abundance of these organisms in a given ecological niche (PARKES, 1987). Phospholipids are not found in storage lipids and have a relatively rapid turnover in sediments so the assay of these lipids provides a measure of the viable cellular biomass when compared to other measures of biomass such as enzyme activities, muramic acid levels, total ATP, and respiratory activity (WHITE, 1983). PLFA are presently considered as most sensitive and highly useful measures to determine the microbial biomass and community structure thus far developed (BOBBIE and WHITE, 1980; GUCKERT *et al.*, 1985; MANCUSO *et al.*, 1990; WHITE *et al.*, 1984). The application of membrane phospholipids to quantify viable cell biomass has been extensively validated for subsurface aquifer sediments (BALKWILL *et al.*, 1988). The advantages of these biochemical analyses have also been reviewed (WHITE, 1988). The present analysis of the fatty acids profile derived from microorganisms adapted to the specific environment would provide an insight into microbial component in the specific environment without further microbial specifications, and particularly a useful information for environmental chemists. Hence the present study was undertaken to understand better, and to describe the microbial components in sediments of the study area.

## MATERIALS AND METHODS

*Study area*

The present study area is located in the southern part of the Seto Inland Sea (131°30′E-132°30′E and 32°30′N-34°30′N) which includes one of the heavily polluted coastal areas in Japan. This part of the Inland Sea has its exit to the Pacific Ocean through the Bungo Channel. Therefore, the tidal current in the Inland Sea is influenced by the incoming tidal waves from Pacific Ocean through the Bungo Channel. YANAGI and OHBA (1985) reported the development of a distinct tidal front in the Bungo Channel of the Inland Sea during summer. Sampling stations (Fig. 1) were selected in the Hyuga Nada (2, 3, 4), Bungo Channel (6, 7, 8) and also from the coastal area (1, 5). To compare the phospholipid ester-linked fatty acid (PLFA) composition in these stations with the northern part of the Seto Inland Sea, the station 9 was selected near Kure (Fig. 1).

*Sample collection*

Samplings in the study area were carried out on board R/V *Toyoshio Maru* from 20 to 22 June 1989. Sediment samples were collected by Smith-McIntier sampler (0.1 $m^2$) from the selected sampling sites of the study area (Fig. 1). The depth of the sampling stations ranged from 20 to 96 m. About 2 cm of the top layer of the sediment was sampled and frozen at −20°C. The frozen sample was lyophilized under reduced pressure before lipid analysis.

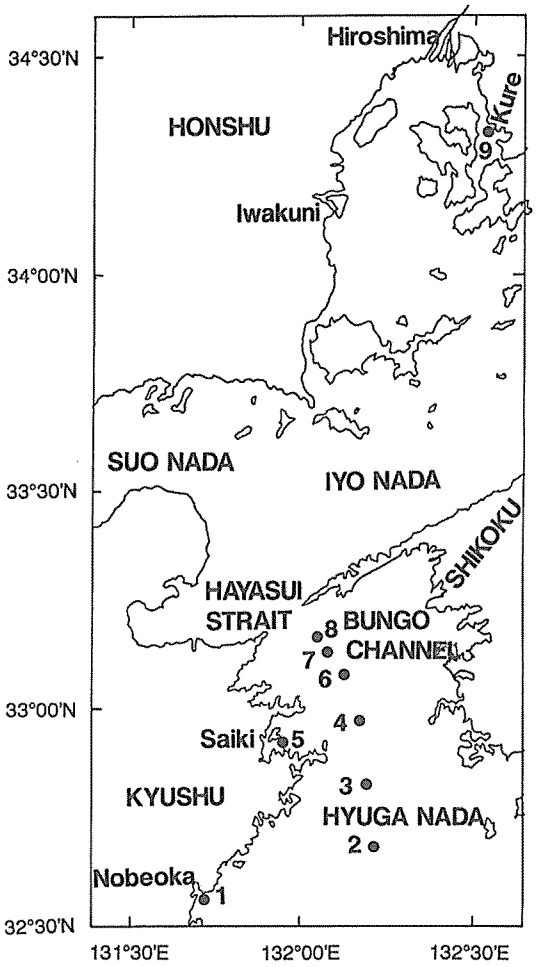

Fig. 1. Location of the sampling stations

*Lipid extraction*

The method of BAIRD and WHITE (1985) was followed for the extraction of lipid from the sediment. Phosphate buffer, chloroform and methanol (2:3:6, v:v:v) were added to the sediment sample in the stainless steel centrifuge tube at approximately 1 ml buffer per gram sediment. The phosphate buffer was prepared by dissolving 8.7 g $K_2HPO_4$ in one liter of distilled water and neutralizing with 1 N HCl to pH 7.4. The tube was closed with teflon-lined stoppers, shaken vigorously, and allowed to stand for 12 h. Sample was then centrifuged at 13,700 *g* for 25 min., and the solvent was transferred to a separatory funnel. Equal volumes of chloroform and water, equivalent to the

original volume of chloroform, were added to the separatory funnel. The separated chloroform layer was recovered and dried at 40°C in a rotary evaporator.

The lipid dissolved in 1 ml of chloroform was fractionated into neutral lipids, glycolipids and phospholipids by column chromatography on silica gel. The phospholipid fraction eluted by methanol was dried under nitrogen and resuspended in 1 ml of methanol and toluene (1:1, v:v). The extract was subjected to mild alkaline methanolysis (WHITE *et al.*, 1979). The resultant organic layer containing fatty acid methyl esters was separated, dried under nitrogen and then redissolved in hexane. Methyl nanodecanoic acid was added as an internal standard.

*Gas chromatography*

Gas chromatography was performed using a Hewlett-Packard 25 m cross-linked 5% phenylmethyl-silicone capillary column (0.2 mm i. d.) in a Hewlett-Packard 5890A gas chromatograph. Operation conditions were as follows: 30 sec. splitless injection at 250°C; He carrier gas with column head pressure, 15 psi; initial column temperature, 80°C, increasing at a rate of 20°C $min^{-1}$ for 3 min, 3°C $min^{-1}$ to 270°C, and isothermal for 10 min. Tentative peak identification, prior to GC-MS analysis, was based on comparison of retention times with those obtained for authentic fatty acid standards (Supelco Inc., U. S. A.). Peak areas were quantified with chromatography software (HP, U. S. A.) operated using a computer. Gas chromatography-mass spectrometry was performed with a Hewlett-Packard 5985B instrument (MS detector) fitted with the same type of column used for gas chromatography. Confirmation of monoenoic double bond position was carried out by GC-MS analysis of the adducts following reaction of the sample with dimethyl disulfide (NICHOLS *et al.*, 1986).

*Nomenclature*

Fatty acids are designated based on number of carbon atoms, number of double bonds, followed by the position of the double bond nearest the d (carboxyl) end of the molecule. When the exact positions of the double bonds were not determined, the d suffix was omitted. The suffixes 'c' and 't' indicate *cis* and *trans* geometry. 'br' indicates the type of branching is undetermined. The prefixes 'i' and 'a' refer to iso and anteiso branching, respectively. The designation 10Me16:0 means methyl branching carbon atom at 10 from the carboxyl end of the molecule. The prefix 'cy' refers to cyclopropane group.

Cluster analysis was performed to determine the similarity of PLFA composition in sediments using a computer (NEC PC 9801 VX).

## RESULTS AND DISCUSSION

*Fatty acid composition*

Analysis of the PLFA in sediment has been postulated as a mean of estimating the different types of bacteria in sediments. The fatty acids in the range of C12 to C19 are known to be bacterial origin (SHAW, 1974) which distinguish them from eukaryotic organisms and in certain cases from each other (LECHEVALIER, 1977). The amounts of individual phospholipid ester-linked fatty acid (PLFA) determined in the sediment samples of the study area are expressed as a percentage of total PLFA for each stations (Table 1). Thirty three individual PLFA were identified in the sediment samples collected in the pre-

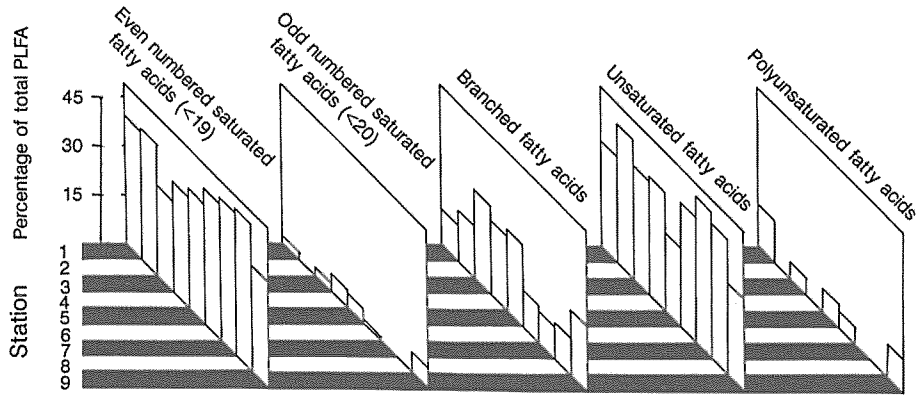


Fig. 2. Different group of PLFA in sediments of the study area

sent study. Total percentages of even and odd numbered straight chain fatty acids with chain length shorter than 20, unsaturated acids, branched acids and acids with chain length longer than 19 are shown in Fig. 2. In all the samples the straight chain PLFA were dominantly present followed by unsaturated fatty acids and branched fatty acids. Generally the polyunsaturated fatty acids (PUFA) and odd numbered straight chain fatty acids were present in low percentages (Fig. 2). The odd numbered saturated fatty acids at stations 2, 7, and 8 and the PUFA at stations 2, 4, 7 and 8 were undetected (Fig. 2). The even numbered PLFA were dominated by 16:0 which is the major sedimentary fatty acid and 18:0 followed by 14:0 and 12:0. The odd numbered saturated acids (15:0 and 17:0) were present in low percentages. The branched PLFA i15:0, a15:0, i16:0, i17:0 and a17:0 were also identified. The branched PLFA are reported to be characteristic acids of bacteria (GILLAN and HOGG, 1984; PERRY *et al.*, 1979), sulfate reducing bacteria (SRB) (EDLUND *et al.*, 1985), gram positive bacteria (KANEDA, 1977) and anaerobic bacteria (WHITE, 1983; EDLUND *et al.*, 1985). The monoenoic acids are characteristically bacterial origin (GILLAN *et al.*, 1983). The presence of straight chain, branched and monoenoic acids in sediments of the present study area suggests the presence of both aerobic and anaerobic bacteria in the study area. These fatty acids were used as markers for bacterial contribution to sediments as these acids are commonly encountered in bacterial lipids (PERRY *et al.*, 1979). The presence of these acids was also reported in mollusks (ACKMAN *et al.*, 1971) and plankton. The fact that these acids are generally found in higher concentrations in bacteria than in other organisms, make them useful indicators of bacterial lipid contribution to sediments. The number of fatty acids determined at stations 1,5, and 9 were higher than the other stations studied (Table 1). Many fatty acids commonly considered to be indicative of bacterial contribution to sediments were identified in the present study.

10Me16:0 fatty acid was identified in significant quantities and its presence in sediments of the study area suggests the contribution from anaerobic bacteria especially sulfate reducing bacteria. The presence of 10Me16:O PLFA without abundance of other methyl branching fatty acids is signature for the SRB *Desulfobacter* (DOWLING *et al.*, 1986; SMITH *et al.*, 1986). The total PLFA of this organism consists of approximately 27% of 10Me16:0 (DOWLING *et al.*, 1986). The fatty acid profile of *Desulfobacter* growing on acetate

was dominated by even numbered, straight chain acids such as 16:0 and 14:0 and contained higher amounts of 10Me16:0 which was not detected in other SRB investigated. The fatty acids i15:0, a15:0, i17:0 and a17:0 are also known to be present in higher amounts in SRB *Desulfovibrio* (BOON *et al.*, 1987; EDLUND *et al.*, 1985). The sediment of the present study are enriched by these marker acids and the greater proportion of these acids reflect the contributions of anaerobic bacteria.

Cyclopropane fatty acids are reported to be bacterial origin and are occasionally reported in sediments (CRANWELL, 1976). The source of these sedimentary cyclopropanoid acids is from bacterial lipids, in which they are often determined, particularly cy19:0 which appears to be unique to bacterial lipids. However in the present study, this acid could be determined in only one sample (Table 1). High concentrations of *cis* fatty acids in aerobic and anaerobic heterotrophs as well as in sediments were reported (PERRY *et al.*, 1979). Although these fatty acids have been reported for other marine organisms, like iso and anteiso branched fatty acids, these acids appear to be present in higher relative percentage in bacteria and hence they are also useful microbial marker acids for the presence of bacteria in sediments (Table 1).

The bottom water at stations 1,5 and 9 was anoxic and the presence of hydrogen sulfide in sediments was also reported. The surface sediment in the coastal area was characterized particularly by the presence of black, silty clay with a strong hydrogen sulfide. Temperature of the study area ranged between 18 and 23°C. The existing anoxic condition also supports the presence of anaerobic bacteria in sediments of the study area.

Fatty acids, which are characteristic of bacteria were used to calculate the similarity of the fatty acid composition among the stations of the study area. The results of the cluster analysis based on fatty acid composition are shown in Fig. 3. Four clusters were obtained and the possible microbial community structure in the study area are known (Fig. 4). Using average linkage clustering procedure, 9 stations were clustered into four which are significant at the 84% similarity level. The inter group similarity values between all clusters varied from 92 to 100% on the basis of the fatty acid composition. The microbial community structure of the sediments as characterized by PLFA profiles were remarkably different in all the stations. With respect to the relative proportions of the isolated fatty acids in the present study, cluster 1 and 2 (stations 5, 9 and 1 respectively) showed that sediments exhibit greater dominance by prokaryotes than other stations as

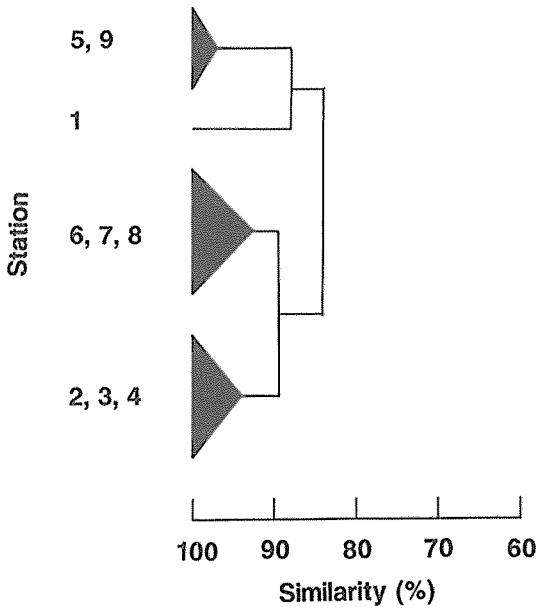


Fig. 3. Simplifed dendrogram based on average linkage method

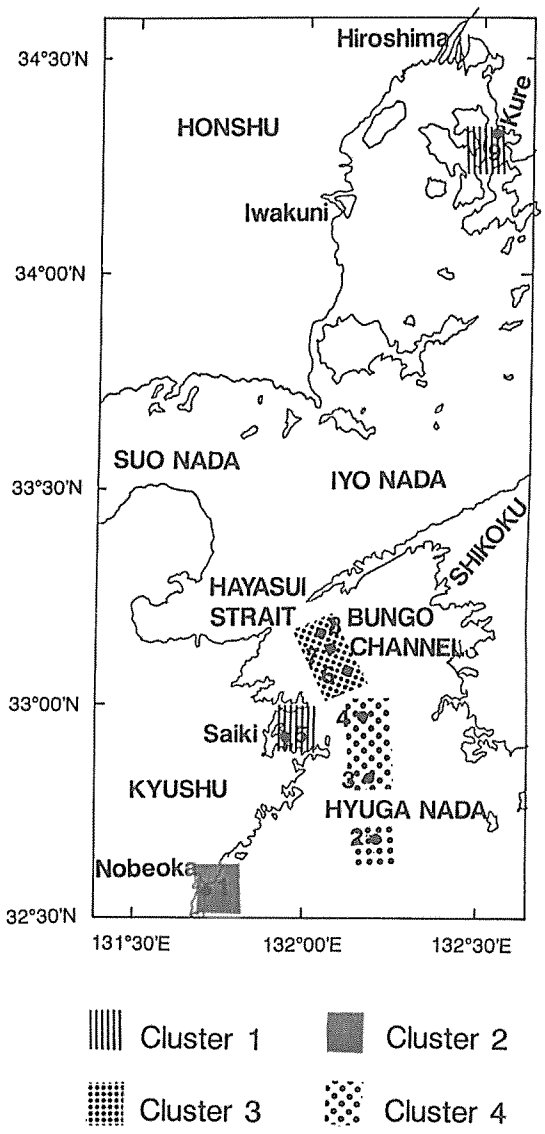


Fig. 4. Distribution of clusters in the study area

characterized by the variation in the PLFA composition at these stations. Several monoenoic acids present in the sediment of these stations were undetected (Table 1) in other stations (2, 3, 4, 6, 7, and 8) which are grouped into clusters 3 and 4.

*Microbial biomass*

Total PLFA per g dry weight sediment was calculated as the sum of all the fatty acids identified in the sediment. Total PLFA concentration varied from 0.33 (station 2) to 2.38 (station 1) $\mu$g/g dry weight sediment (Table 2). The total PLFA content and the calculated biomass (described below) showed variation which may be due to the sediment nature in the study area. Sand and sandy-silt were dominant in sediments of stations 2, 3, 4, 6, 7, and 8 which is influenced by wind current and tidal current in the north and south of Hayasui strait (YANAGI, 1990).

The concentration of PLFA in sediment was used to calculate approximate microbial biomass in the sediment. The average bacterium, the size of *E. coli,* contains 100 $\mu$mol PLFA/g dry weight and 1 g of bacteria is equivalent to $5.9\times10^{12}$ cells (BALKWILL *et al.,* 1988; WHITE *et al.,* 1979) which provides $1.7\times10^{-17}$ mol PLFA/bacterial cell (MANCUSO *et al.,* 1990). Microbial biomass thus calculated using these conversion factor in the present study area showed that the bacterial biomass in sediments ranged from $1.5\times10^{7}$ cells/g dry weight at station 2 to $9.3\times10^{7}$ cells/g dry weight at station 1 (Table 2). The calculated biomass showed variations among the stations of the present study area. The calculated values of biomass based on PLFA concentration may underestimate the actual number of cells if the cells present are smaller than *E. coli.* Nevertheless, it was believed, in spite of these considerations that such approach will highlight PLFA differences in terms of contributing microbial community.

The ratios of iso and anteiso 15:0 to 16:0 in sediment are shown in Table 2. Branched fatty acids are characteristically bacterial origin (GILLAN and HOGG, 1984). The sum of the

Table 1. Percentage composition of PLFA in sediment samples

| Fatty acid | Station: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 12:0 | 0.59 | — | — | — | — | — | — | — | 1.22 |
| 14:0 | 11.74 | — | 3.83 | 4.94 | 4.32 | 5.49 | 8.77 | 10.38 | 7.20 |
| 15:0 | 1.52 | — | 2.84 | 4.09 | 3.57 | 1.22 | — | — | 5.50 |
| 16:0 | 22.74 | 40.91 | 24.77 | 29.22 | 25.52 | 29.57 | 35.56 | 35.78 | 19.39 |
| 17:0 | 0.89 | — | — | 1.76 | 1.53 | — | — | — | 1.20 |
| 18:0 | 4.74 | — | — | — | 7.86 | 7.61 | — | — | 5.45 |
| 24:0 | — | — | — | — | 1.63 | — | — | — | 1.90 |
| a12:0 | 0.53 | — | — | — | 1.98 | — | — | — | 0.88 |
| i14:0 | — | — | — | — | — | — | — | — | 1.15 |
| br-15:0 | — | — | — | — | — | — | — | — | 0.95 |
| i15:0 | 3.25 | 15.75 | 12.80 | 10.77 | 9.41 | 8.34 | 7.33 | 11.59 | 6.26 |
| a15:0 | 1.05 | — | 6.68 | 4.55 | 3.98 | — | — | — | 5.14 |
| i16:0 | 3.34 | — | 3.14 | 1.84 | 1.61 | 1.96 | — | — | 2.39 |
| 10Me16:0 | 1.90 | — | 4.93 | 5.86 | 5.12 | 1.17 | 2.58 | — | 2.48 |
| i17:0 | — | — | — | — | 1.46 | — | — | — | 0.72 |
| a17:0 | — | — | — | — | — | — | — | — | 0.65 |
| br-19:1 | — | — | — | — | 1.68 | — | — | — | 2.24 |
| cy19:0 | 0.78 | — | — | — | — | — | — | — | — |
| 16:1d7 | 1.89 | — | — | — | — | 1.95 | — | — | 1.35 |
| 16:1d9*c* | 15.55 | 20.17 | 12.08 | 10.17 | 8.88 | 21.47 | 15.67 | 11.63 | 8.85 |
| 16:1d9*t* | 2.78 | — | — | — | — | 2.86 | — | — | 1.24 |
| 16:1d11*c* | 0.66 | — | — | — | — | — | — | — | 1.73 |
| 17:1 | — | — | — | — | — | — | — | — | 2.56 |
| 17:1d9 | 0.40 | — | — | — | — | — | — | — | — |
| 18:2 | 2.25 | — | — | 3.62 | 3.16 | 2.66 | — | — | 1.77 |
| 18:1d9*c* | 6.09 | — | 6.82 | 7.88 | 6.88 | 4.18 | 12.40 | 12.76 | 4.92 |
| 18:1d9*t* | 3.55 | — | 10.50 | 6.30 | 5.50 | 5.88 | 7.32 | — | 4.51 |
| 18:1d11 | — | 23.18 | 6.10 | 9.00 | — | — | 10.36 | 17.86 | — |
| 20:5 | 1.24 | — | — | — | — | — | — | — | 0.88 |
| 20:4 | 10.01 | — | 2.74 | — | 2.02 | 2.84 | — | — | 3.14 |
| 20:1d11 | — | — | — | — | 1.61 | — | — | — | 1.88 |
| 22:1 | 1.59 | — | 2.76 | — | 2.28 | 2.80 | — | — | 2.44 |
| 22:1 | 0.92 | — | — | — | — | — | — | — | — |

relative abundance of iso and anteiso isomers of 15:0 represents the bacterial component of the community (NICHOLS *et al.*, 1987). 16:0, a PLFA is found ubiquitously in most organisms. The ratio of iso and anteiso 15:0 to 16:0 in samples will provide an indication of the proportion of bacteria (MANCUSO *et al.*, 1990). These ratios for the sediment samples of the present study area ranged from 0.19 to 0.79 implying a 4-fold increase in the relative abundance of bacterial signature acids in the study area.

The relative abundance of branched fatty acids were generally higher in all the samples than the acids with chain length longer than 20. Branched acids contributed 10 to 27% to the total PLFA whereas the polyunsaturated acids made up only 0 to 14% in these samples (Fig. 2). The polyunsaturated acids 20:5, 20:4, 20:1 and 22:1 isolated from the sediment samples of the present study area are characteristics of microeukaryotes and accounted for

Table 2. Total PLFA content and microbial biomass in sediments

| Station | Total PLFA (μg/g) | Biomass (×10⁷ cells) | i+a15:0 /16:0 | *t/c* 16:1d9 |
|---|---|---|---|---|
| 1 | 2.38 | 9.3 | 0.19 | 0.18 |
| 2 | 0.33 | 1.5 | 0.38 | —* |
| 3 | 0.55 | 2.4 | 0.79 | — |
| 4 | 0.64 | 2.9 | 0.52 | — |
| 5 | 0.73 | 3.1 | 0.52 | — |
| 6 | 0.69 | 3.0 | 0.28 | 0.13 |
| 7 | 1.10 | 5.0 | 0.21 | — |
| 8 | 0.48 | 2.2 | 0.32 | — |
| 9 | 1.70 | 6.9 | 0.59 | 0.14 |

**trans* acid was not detected

low percentage in the total PLFA. The relative variations in PUFA and branched PLFA among the sediment samples are an indication of the microeukaryotic and bacterial components of the sediment microbial community. The virtual absence of PUFA in some stations suggests the decreased input from microeukaryotes or zooplankton. However the presence of polyunsaturated 18 carbon acids in sediments may have source from plankton as reported by VOLKMAN and JOHNS (1977). Further the sediment in the Bungo Channel and Hyuga Nada is under direct influence of tidal current. Coastal sediments are exposed to significant wave action and tidal action. Wave generated hydrostatic pressure has been known to increase water soluble transport, increasing oxygen penetration into sediments (WEBB and THEODOR, 1972). However the results of dissolved oxygen of the present study area revealed the reduced availability of oxygen in the overlying waters of sediments and existence of anoxic conditions in the sampling area may have caused the loss of microeukaryotic biomass, the decrease in aerobic bacteria and the increase in SRB and other anaerobic bacteria. FINDLAY *et al.* (1990) reported that decrease in oxygen level in the sediment will reduce the contribution of biomarker acids by the microeukaryotes.

The ratios of *t/c* of 16:1d9 are also given in Table 2. *Cis* isomers of monounsaturated PLFA are commonly present in microbial cellular components (GUCKERT *et al.*, 1987). High abundances of *trans* isomers have been recently detected in cultures of *Pseudomonas atlantica, Vibrio cholerae,* and environmental enrichments, and have been associated with strategies for survival during physiological stress in these organisms (GUCKERT *et al.*, 1985, 1986, and 1987; NICHOLS *et al.*, 1986; RAJENDRAN *et al.*, 1990). The ratios of *trans* and *cis* (*t/c*) in most bacterial and sediment samples were determind to be less than 0.1 (GILLAN and HOGG, 1984; GUCKERT *et al.*, 1985; NICHOLS *et al.*, 1986; PERRY *et al.*, 1979) and greater than 1 have been determined during starvation. GUCKERT *et al.* (1986) suggested that the ratio of *trans* and *cis* acids may be useful as an indicator to determine the nutritional status of bacteria in the aquatic environment. In the present study, the *t/c* ratio for 16:1d9 in sediments collected at stations 1, 6, and 9 were 0.18, 0.13, and 0.14, respectively. The *trans* isomer of 16:1d9 was undetermined in other samples.

From the results of the present study, it could be concluded that the aerobic and anaerobic bacteria were predominantly present at stations 1, 5, and 9 as evidenced by the presence of branched and monounsaturated fatty acids, whereas in other stations, the absence of many marker fatty acids suggest that microbial biomass is low.

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# 沿岸海底堆積物中における微生物バイオマーカー脂肪酸の組成

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沿岸海底堆積物中の微生物現存量と微生物の群集構造を明らかにするために堆積物中のリン脂質の脂肪酸組成を解析した。その結果，33種の脂肪酸が検出され，それらの中には多くの細菌指標性の脂肪酸が含まれていた。微生物現存量は堆積物乾重量 1 g あたり $1.5\times10^7$～$9.3\times10^7$ cells と推定された。微生物バイオマーカー脂肪酸の類似度分析からは微生物の群集構造に対応する 4 つの異なったクラスターが認められた。沿岸堆積物中のリン脂質脂肪酸中の不飽和脂肪酸と分枝脂肪酸の分析結果から好気性細菌ならびに嫌気性細菌，特に硫酸塩還元細菌の地域的な存在特性が明らかとなった．