The Microbiology of the Terrestrial Deep Subsurface

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Utility of the Signature Lipid Biomarker Analysis in Determining the In Situ *Viable Biomass, Community Structure, and Nutritional/Physiologic Status of Deep Subsurface Microbiota*

D. C. White and D. B. Ringelberg

CONTENTS

KEY WORDS: *signature lipid biomarkers, viable biomass, community composition, physiological status,* in situ *analysis, nonculturable microbes, detection of viable biomass, phospholipid ester-linked fatty acids, poly beta-hydroxy alkanoate, stress biomarkers, unbalanced growth, toxicity response.*

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8.1 Introduction

The classical microbiological approach which was so successful in public health for the isolation and culture of pathogenic species is clearly less than satisfactory for subsurface sediment samples. It has been repeatedly documented in the literature that viable or direct counts of bacteria from various environmental samples may represent only 0.1 to 10% of the extant community (White et al., 1993; Tunlid and White, 1991; White, 1983, 1986, 1988). Moreover, microbes may still be metabolically active and potentially infectious even though they are not culturable. Classical microbial tests are time-consuming and provide neither an indication of the nutritional status nor evidence of toxicity within the extant community.

The signature lipid biomarker (SLB) analysis does not depend on growth or morphology for identification. Instead, microbial biomass and community structure are determined in terms of universally distributed lipid biomarkers which are characteristic of all cells. Lipids, recoverable by extraction in organic solvents, are an essential component of the membranes of all viable cells. SLB have been shown to be readily extractable from most environments, including the deep subsurface. The extraction process provides for both purification and concentration of "signature" lipid biomarkers from the cell membranes and walls of microorganisms (Guckert et al., 1985).

8.1.1 Viable Biomass

A determination of the total ester-linked phospholipid fatty acid (PLFA) content provides a quantitative measure of the viable or potentially viable biomass. A viable organism will have an intact membrane containing PLFA. Upon cell death or a cell lysis, cellular enzymes hydrolyze phospholipids releasing the polar head groups. The hydrolysis can occur within minutes to hours of cell lysis (White et al., 1979). The lipid moiety remaining which is called a diglyceride, contains the same signature fatty acids as the phospholipids (Figure 8.1). An estimation of the total nonviable and the total viable biomass can be made by measuring diglyceride fatty acids and phospholipid fatty acids, respectively. A careful study of subsurface sediment showed that the viable biomass, as determined by PLFA, was equivalent (with a smaller standard deviation) to estimations based on intercellular ATP, cell wall muramic acid, and carefully performed acridine orange direct counts (Balkwill et al., 1988). Since phospholipids are found in reasonably constant amounts in all cellular membranes in a variety of bacterial cells, have a high natural turnover rate, and are rapidly degraded in nonviable cells, the measurement of PLFA provides an accurate estimation of the viable or potentially viable microbial biomass.

8.1.2 Community Structure

The analysis of SLB by capillary gas chromatography/mass spectrometry (GC/MS) provides sufficient information for the identification of specific subsets of the microbial community. Specific groups of microbes often contain characteristic lipids, in particular, fatty acids (White et al., 1993; Tunlid and White, 1991; White, 1983, 1986, 1988). For example, PLFA prominent in the hydrogenase-containing *Desulfovibrio* sulfate-reducing bacteria are distinctly different from those found in the *Desulfobacter* sulfate-reducing bacteria (Edlund et al., 1985; Dowling et al., 1986). The SLB analysis is

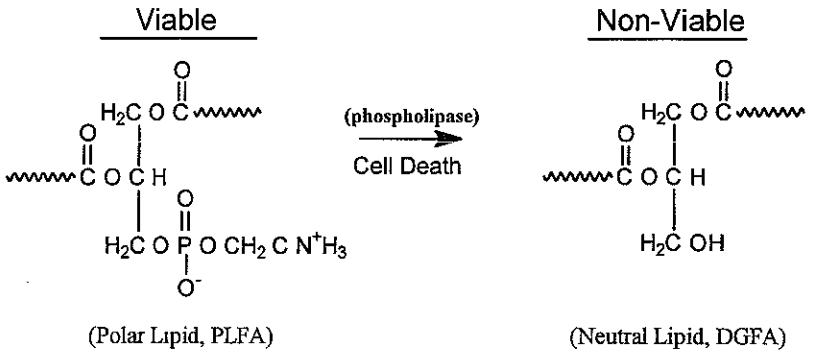

FIGURE 8.1
Illustration showing the conversion of a phospholipid (PL) to a diglyceride (DG) as a result of cell death.

unable to identify every species of microorganism in an environmental sample as many species contain overlapping PLFA patterns. However, many species or physiologically similar groups of microorganisms are readily detected in environmental samples. Species such as *Francisella tualrensis, Vibrio cholera, Syntrophomonas wolfei, Flavobacterium* spp., members of the genera *Vitreoscilla, Flexibacter, Filibacter, Thiobacilli, Frankia,* and groups of organisms such as the Archae, actinomycetes, acetogens, and type I and type II methanotrophs have all been detected in complex environmental matrices by analyses of the respective PLFA patterns (Nichols et al., 1985, 1986, 1987; Guckert et al., 1986; Kerger et al., 1986, 1987; Ringelberg et al., 1988; Phelps et al., 1991; Coleman et al., 1993; Tunlid et al., 1989).

Distinctive patterns of fatty acids (largely from phospholipids and lipopolysaccharides) released from cultured bacteria are currently used for pure culture identifications or to group them by fatty acid relatedness. Descriptive patterns of prominent ester-linked fatty acids recovered from isolated microbes grown on standardized media have been developed for over 650 species (Microbial Identification System, MIDI, Newark, DE) (Welch, 1991). Patterns of PLFA can also be used in identifying single species. For example, a comparison of PLFA patterns of 18 species of methane-oxidizing bacteria and 21 species of Gram-negative sulfate-reducing bacteria by hierarchical cluster analyses paralleled the relationships obtained from the analysis of 16S rRNA sequence homologies (Guckert et al., 1991; Kohring et al., 1994). Since identifications are based on the isolation and culture of microorganisms, unculturable microbes go undetected.

The analysis of other lipids such as sterols for microeukaryotes (nematodes, algae, protozoa) (White et al., 1980), glycolipids for Gram-positive bacteria and phototrophs, and hydroxy fatty acids from the Lipid A portion of a lipopolysaccharide (LPS-OHFA) for Gram-negative bacteria (Parker et al., 1982) can provide a more detailed analysis of the microbial community structure (Figure 8.2). Species of the genera *Planctomyces, Pirella, Geobacter, Desulfomonile,* and *Legionella* have all been readily detected and differentiated by analysis of their LPS-OHFA (Kerter et al., 1988; Lovely et al., 1992; Ringelberg et al., 1993; Walker et al., 1993). In a specific application, LPS-OHFA was utilized to differentiate between contamination of sediments by an enteric bacterium from that by a pseudomonad (Parker et al., 1982).

The SLB analysis provides a pattern of PLFA which characterizes the entire microbial community. By quantifying differences between PLFA patterns obtained from environmental samples, inferences can be made regarding shifts in community composition. Multivariant statistical analyses, such as hierarchical cluster and principal components analysis, have proven to be valuable tools in identifying these differences.

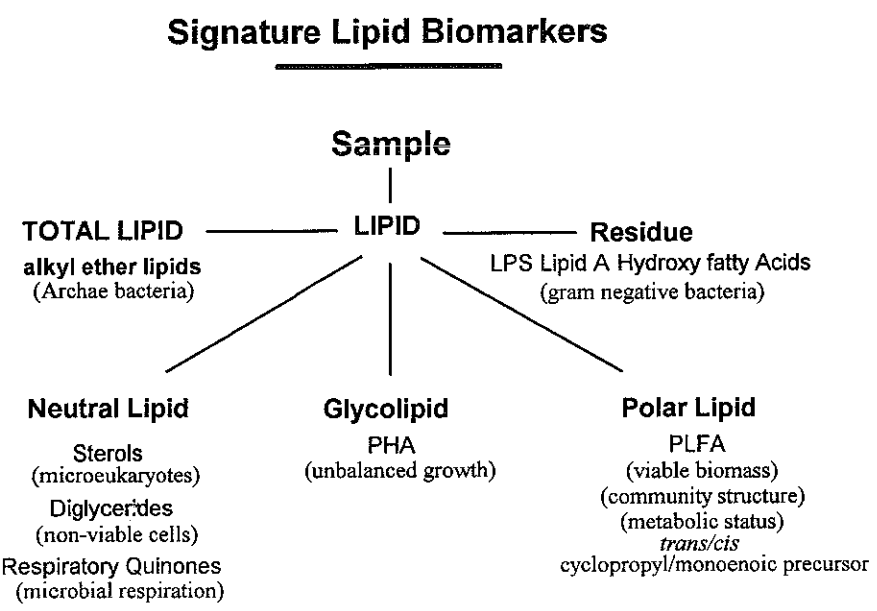

FIGURE 8.2
Schematic representation of the signature lipid biomarker analysis.

Variance among a specific PLFA or group of PLFA within the sample set can then be interpreted based on what is currently known regarding fatty acid biosynthesis and bacterial membrane PLFA compositions.

8.1.3 Nutritional/Physiological Status

In bacteria, specific patterns of PLFA can indicate physiological stress. Exposures to toxic environments can lead to minicell formation and a relative increase in *trans* monoenoic PLFA, when compared to the *cis* homologues. it has also been shown that for increasing concentrations of phenol toxicants, the bacteria *Pseudomonas putida* forms increasing proportions of *trans* PLFA (Heipieper et al., 1992). The ratio of *trans/cis* PLFA in toxic sediments fluctuates around 0.2, and in rapidly growing "healthy" bacteria it is around .01. Similarly, ratios of cyclopropane fatty acids to their monoenoic homologues (as well as *trans/cis* ratios) were shown to increase in response to a change in environmental conditions, i.e., starvation (Guckert et al., 1986; Kieft et al., 1994) (see Chapter 11).

In addition to PLFA, other lipid components provide insight into a microbial community's nutritional or physiological status. The accumulation of poly β-hydroxyalkanoic acids (PHA) in bacteria (Nickels et al., 1979) and triglycerides in microeukaryotes (Gehron and White, 1982), when compared to PLFA concentrations, are two such compounds. Bacteria exposed to adequate carbon and terminal electron acceptors form PHA when they are unable to divide. Under ambient environmental conditions, a cessation of cell division is generally due to the absence (or lack of) an essential nutrient or nutrients (phosphate, nitrate, trace metal, etc.).

The analysis of the respiratory quinone structure provides an estimation of the degree of aerobic microbial activity (Figure 8.3) (Hedrick and White, 1986). Environments with high potential terminal electronic acceptors (oxygen, nitrate) induce the formation of benzoquinones in bacteria whereas bacteria respiring on organic substrates form naphthoquinones. Fermentative organisms, such as many *Lactobacillus*

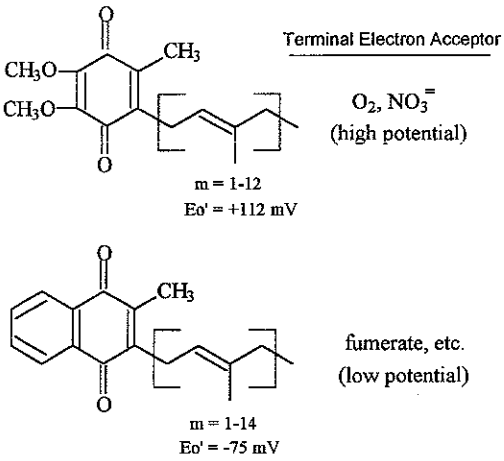

FIGURE 8.3
Illustration of bacterial respiratory quinone structure where high potential terminal electron acceptor refers to benzoquinones and Gram-negative bacteria, and low potential to menaquinones and both Gram-negative and Gram-positive bacteria.

sp., may actually form no respiratory quinones. By determining the quinone content within a given sample, proportions of aerobic, anaerobic respiratory, and/or anaerobic fermentative bacteria can be estimated. When compared to viable biomass estimates and community structure determinations the analysis of respiratory quinones, polyhydroxy alkanoates, and the ratios of specific PLFA (*trans/cis* and cyclopropyl to monoenoic precursor) provide valuable insights into the nutritional and physiological status of the extant microbiota.

8.2 Materials and Methods

8.2.1 Lipid Analyses

Frozen or lyophilized cells from subsurface isolates or sediments and/or filter retentates from ground waters can be quantitatively extracted for PLFA and other lipid components. Prior to extraction, sediments are homogenized under sterile conditions. Samples are saturated with a single-phase organic solvent system comprised of chloroform, methanol, and aqueous 50 mM PO_4 (pH 7.4) buffer (1:2:0.8, v:v:v) (White et al., 1979). After agitation and an extraction period of approximately 3 h in duration, equal volumes of chloroform and nanopure water are added to the extractant, resulting in a two-phase system with a final composition of 1:1:0.9, chloroform:methanol:water, v:v:v. In the case of the sediments, the samples are centrifuged (~650 × g for 30 min) and the supernatant decanted into a separatory funnel prior to the addition of chloroform and water. The chloroform is used in washing the sediment, followed by recompaction, prior to combination with the original extractant and water in the separatory funnel. A complete separation of phases usually occurs within a 12-h period. The lower organic (lipid-containing) phase is then collected and evaporated at 37°C either under a gentle stream of N_2 or by rotary evaporation. the upper phase and residue (lipopolysaccharide) are also collected for analysis of the LPS-OHFA as described by Parker et al. (1982). The organic (lipid-containing) phase is then fractionated on a

silicic acid column into neutral lipids, glycolipids, and polar lipids (Tunlid et al., 1989). The polar lipid fraction, recovered in methanol, is subjected to a transesterification for the recovery of PLFA (Tunlid et al., 1989). PLFA are then further separated and quantified by GC/MS with selected ion recording using either positive ion chemical (Tunlid et al., 1989) or electron impact ionizations (Kieft et al., 1994). The neutral lipid fraction may be processed for the collection of diglyceride fatty acids (Kieft et al., 1994), triglycerides (Gehron and White, 1982), and sterols (Nichols et al., 1983). The glycolipid fraction may be processed for the collection of PHA (Findlay and White, 1983) and other complex carbohydrates such as those found in cell walls of certain Gram-positive bacteria.

8.2.1.1 Fatty Acid Nomenclature

Fatty acids are designated by the total number of carbon atoms followed by the total number of double bonds beginning with the position of the double bond closest to the methyl end (ω) of the molecule. The configuration of the double bond is designated by either a c for *cis* or t for *trans*. For example, 16:1ω7c is a PLFA with a total of 16 carbons, 1 double bond located 7 carbons from the methyl end of the molecule in the *cis* configuration. Branched fatty acids are designated as i for *iso*, a for *anteiso*, if the methyl branch is one or two carbons from the ω end of the molecule (i.e., i15:0 or a15:0), or by a number indicating the position of the methyl group from the acid end of the molecule (i.e., 10me16:0). Methyl branching at undetermined positions is indicated by a "br". Cyclopropyl fatty acids are designated by the prefix "cy". For hydroxy fatty acids, the position of the hydroxyl group is numbered from the acid end of the molecule followed by the prefix "OH" (i.e., 3-OH14:0).

8.2.1.2 Statistical Analyses

Typically, results of a PLFA analysis are expressed as a molar percentage and subjected to an analysis of variance to address differences between population means. The same PLFA profiles can also be treated as multivariant data. Applications such as hierarchical cluster and principal components analyses often provide information regarding community shifts which were not identified in an ANOVA. It is generally accepted that the multivariant statistics be performed on ratios and that the values be arcsin transformed prior to analysis. A hierarchical cluster analysis will provide a dendrogram which illustrates linkages between samples based on similarities in PLFA profiles. The significance of these similarities (usually expressed as Euclidean distances) is dependent on the size and nature of the sample set being analyzed. A principal component analysis reduces a number of correlated variables into only a few mutually independent variables (i.e., principal components). Results of the analysis are generally plotted in two-dimensional space and indicate not only how samples vary in PLFA composition, but also to what extent each PLFA accounts for this variance. Comparisons of the PLFA which describe specific subsets of samples can then be used in assessing shifts in microbial community composition (Frostegaard et al., 1993).

8.2.1.3 Procedural Considerations

In order to detect PLFA extracted from microbial cells recovered from the deep subsurface, it is necessary that background levels of PLFA (those introduced in sample workup) be kept at a consistently low level. Since the sample size is often restricted when working in the deep subsurface, optimizing the signal-to-noise ratio becomes necessary. In order to do so, it is usually necessary to obtain the highest-purity

solvents and chemicals available. In addition, all glassware should be cleaned and muffled in a furnace at ~450°C for a minimum of 4 h prior to use. Care must be taken at all times to minimize the introduction of lipids during sample workup. Some common sources of contamination are the skin, plastics, condensation, vacuum grease, and pump oil vapors. By establishing laboratory practices which check solvent quality on a routine basis and incorporate procedural blanks with each set of samples processed, sources of contamination can be more quickly identified and eliminated, minimizing damage to the research results.

8.3 Results and Discussion

8.3.1 Establishment of Uncontaminated Deep Subsurface Samples

The recovery of sediments from the deep subsurface entails the use of much more complex drilling procedures than those employed in collecting samples from shallow aquifers (see Chapter 3). In many instances drilling muds and make-up waters are required. The utilization of drilling muds under high pressure imposes a great challenge in assuring the quality of samples recovered from the deep subsurface. In 1986 the Department of Energy Science Program began an interdisciplinary program (Wobber) to develop the technology necessary for the recovery of deep subsurface samples suitable for microbiological analysis. The technology that resulted carefully defined the levels of possible contamination through the innovative use of tracers and the control of both drilling and sample handling procedures (Phelps et al., 1989; Russell et al., 1992; Colwell et al., 1992).

SLB proved to be particularly useful in establishing the validity of microbial communities recovered from deep subsurface sediments (Lehman et al., 1996). Table 8.1 highlights differences in PLFA abundance among samples of drilling muds, make-up waters, corehole cuttings, and sample cores recovered from a site on the eastern coast of the U.S. Samples of drilling mud contained between 3 to 15 times more viable biomass than that recovered form the sample core. The sample core itself resulted after three consecutive parings of outer materials. Analyses of PLFA content of each of these parings showed biomass to progressively increase from the outside in (Lehman et al., 1996). Analysis of the PLFA profiles showed a concurrent increase in the proportions of terminally branched saturated PLFA, with the highest proportion occurring in the sample core (Lehman et al., 1996). Since there were substantial differences in both viable biomass and community composition between the outer paring and the inner core material, it was difficult to conceive of how there could have been significant microbiological contamination from the drilling muds. A comparison of PLFA mole percent profiles representative of drilling muds, make-up waters, and cuttings and cores by hierarchical cluster analysis showed that distinct differences in PLFA composition existed between the different sample types (Figure 8.4). There was a remarkable parallel between the result of this cluster analysis and that generated through the application of a community-level physiological profile (CLPP) (Lehman et al., 1996). This profile is based on the results of a sole-carbon-source utilization test using 95 different sole-carbon sources (and nutrients). The parallelism between the two assays reinforces the assumption that there was essentially no transference of microbial contamination from the drilling muds into the sample cores. Used together or separately, the two assays, best described as inherent traces, provided an additional and valuable measure of sample microbiological integrity.

TABLE 8.2

Comparison of PHA/PLFA Ratios in Whole and Homogenized Subsurface Sediments Over a 32-Week Incubation Period

Time[a]	Whole[b]	Homogenized[c]
3	0.05 ± 0.09	0.02 ± 0.03
5	0	0
9	0.01 ± 0.00	0
16	10.7 ± 13.5	5.45 ± 9.45
32	66.7 ± 112	175 ± 275

Note: Values expressed as an average of the ratio of PHA/PLFA ± a standard deviation.

[a] Time expressed in weeks.

[b] Intact sediment cores.

[c] Sediment cores homogenized with mortar and pestle and sieved (2 mm).

TABLE 8.3

Viable Microbial Biomass and Community Structural Shifts With Time in Whole (Intact) and Homogenized Sediment Cores Incubated at 15°C

	Biomass[b]		Monosaturates		Terminally Branched Saturates	
Incubation[a]	Whole[c]	Homogenized[d]	Whole	Homogenized	Whole	Homogenized
3	0.5 ± 0.4	1.1 ± 1.4	2.9 ± 1.4	2.5 ± 1.8	1.9 ± 2.4	2.1 ± 2.0
5	0.6 ± 0.1	0.6 ± 0.6	5.8 ± 1.4	1.8 ± 1.9	13.6 ± 11.2	3.3 ± 4.0
9	0.6 ± 0.3	0.7 ± 0.6	7.4 ± 1.4	2.2 ± 3.1	25.6 ± 7.2	17.2 ± 20.6
16	4.3 ± 3.7	1.5 ± 0.6	6.6 ± 0.8	2.3 ± 2.1	43.3 ± 8.0	5.2 ± 5.5
32	2.0 ± 1.8	0.3 ± 0.2	4.8 ± 1.7	0.9 ± 1.5	35.9 ± 19.4	9.6 ± 12.2

Note: Values expressed as average ± a standard deviation (n = 3).

[a] Incubation time in weeks at 15°C.

[b] Biomass expressed as picomole PLFA per gram.

[c] Whole indicates an intact core.

[d] "Homogenized" indicates a core after grinding by mortar and pestle and sieving (2 mm).

provided by F.J. Brockman, Battele/PNL). The ratio of PHA/PLFA indicated that with the increase in biomass and changes in community composition, a portion of the extant microbiota was experiencing unbalanced growth. This imbalance was likely due to nutrient limitations, a characteristic of most subsurface environs (Table 8.3). This study demonstrated that changes in the microbiota can occur in intact sediment cores over time and that the point in time a sample is cultured may dictate the results obtained.

Samples of deep subsurface volcanic rock showed large changes in the numbers of viable organisms that were recoverable following 1 week of storage at 4°C and perturbation (Haldeman et al., 1994). Morphologically distinct colonies recovered before and after the incubation were analyzed for fatty acid content and API rapid NFT strips. The analyses showed that while recoverability increased, diversity generally decreased, and that those cultures appearing postincubation showed a greater ability to utilize the carbon sources. The changes in community composition and the fact that some isolates were only recovered postincubation suggested that bacterial outgrowth had occurred. Again, the time at which the cores were sampled resulted in a different description of the extant microbiota.

8.3.2 Near Surface and Deep Subsurface Microbial Communities

8.3.2.1 Near Surface Microbial Communities

Initial applications of SLB methods to subsurface aquifer materials were with near surface fine clays recovered from both vadose and saturated zones from sites in TX, OK, and FL (Smith et al., 1986; White et al., 1983). Results showed that the subsurface microbiota contained intact cytoplasmic membranes composed of PLFA, thus indicating the presence of viable cells. Results also indicated that the abundance of viable subsurface microorganisms were sparse when compared to surface soils. In terms of community composition, the subsurface microbiota was characterized by a greater proportion of Gram-positive and Gram-negative anaerobic bacteria, as compared to surface soils or to estuarine sediments. This was indicated by the detection of an increased abundance of terminally branched saturated PLFAs. In addition, there was an absence of SLB for microeukaryotic organisms (i.e., algae, protozoa, fungi, and micrometazoans) in the subsurface cores. This was indicated by the absence or low abundance of polyunsaturated PLFA. With respect to microbial nutritional status, the subsurface microbiota was characterized by a higher propensity for unbalanced growth, as indicated by greater ratios of PHA/PLFA when compared to the surface soils. Surface soils and subsurface sediments, even those collected from near the surface, contained distinctive characteristics which were identified with the SLB analyses. The characteristics identified in the studies in TX, OK, and FL have also been identified at other sites. Different characteristics between surface and subsurface microbiology are, however, likely to occur over large areas.

8.3.2.1.1 Effects of Aquifer Contamination on Microbial Communities

Samples acquired from near surface aquifers contaminated with biodegradable compounds like phenols and creosols showed distinct and reproducible differences in microbial community composition and physiological status between uncontaminated and contaminated sediments (Smith et al., 1986). The differences were attributed to specific responses by the extant microbiota to the presence of the biodegradable contaminant. The contaminated sediments were characterized by an increase in the total viable biomass, a shift in the microbial community composition toward Gram-negative heterotrophs, and a decrease in microbial-related environmental stress. These assumptions were based on the detection of greater total PLFA abundance, an increased percentage of monounsaturated and normal saturated PLFA, and a decrease in the ratios of cyclopropane PLFA to the monounsaturated analogues.

In two integrated DOE bioremediation demonstration programs involving either petroleum (Walker and Walker, 1994) or trichloroethylene (TCE) (Pfiffner et al., 1995) contamination, SLB analyses showed that an increase in microbial abundance occurred as a result of treatments involving nutrient additions. Microbial community shifts were also detected in each study which were related to both the presence and nature of the contaminant. For example, SLB specific for type II methanotrophs were observed to increase after methane gas additions in ground waters collected from the TCE-contaminated site. Similar results were recorded with exposures to propane and air (Ringelberg et al., 1988). Results such as these suggest that the SLB analyses can be used in predicting the success of *in situ* bioremediation when correlations can be made with successful feasibility studies. In addition, SLB analyses provide evidence for bioremediation effectiveness through the monitoring of changes in microbial community composition and abundance (see Chapter 14).

TABLE 8.4

A Comparison of Microbial Biomass in Surface, Near Subsurface, and Deep Subsurface Sediments Recovered in the U.S.

Site	Surface[a]	Near Subsurface[b]	Deep Subsurface[c]
ID	140		2–7
OK	4180	180	
SC	2400		4–12
TX		600	
WA	145		1–16

Note: Values expressed as picomoles PLFA per gram.

[a] Collected from a depth of 0–10 cm.

[b] Collected from a depth of 4 m.

[c] Collected from a depth of >5 m.

8.3.2.2 Deep Subsurface Microbial Communities

Deep subsurface sediments recovered from the two sites in the arid western U.S. (ID and WA) (Colwell et al., 1992) and one in the southeastern coastal plain (SC) (White et al., 1991) showed very low numbers of microorganisms — considerably lower than those observed in near subsurface samples (Table 8.4). A multivariant statistical analysis of the PLFA profiles recovered from these same sediments showed three distinct groups, indicating differences in microbial community composition (Figure 8.5). As defined, group 1 contained the surface samples from all three sites. The PLFA profiles from these samples were enriched in PLFA biomarkers characteristic of soil Actinomycetes (i.e., 10me18:0 or tuberculosteric acid). Also included in this group were the higher biomass (>8 pmol PLFA per gram) vadose zone samples from both the ID and WA sites. Loadings from the analysis indicated that 16:1ω7c and 18:1ω7c were significant in defining group 1. These two PLFA are end products of bacterial anaerobic desaturase fatty acid biosynthesis which is a pathway typically used by Gram-negative bacteria, including the aerobic Gram-negative bacteria which were prevalent in the surface soils. As defined, group 2 was comprised of the majority of deep subsurface vadose zone sediments from the two western sites. These samples were characterized by a very low microbial biomass. The samples collected from around the water table (indicated by the *) showed evidence of toxicity. The *trans/cis* ratios of these samples were greater than 0.1 proposed threshold (Guckert et al., 1986). As defined, group 3 was largely comprised of the SC site samples. Results of the principal components analysis showed two PLFA, 14:0 and 18:2ω6, to be significant in the definition of this group. The dienoic, 18:2ω6, is a prominent fatty acid in the cell membranes of fungal species, whereas the normal saturate, 14:0, has been shown to be prominent in a number of species of microalgae. Although neither fatty acid alone indicates the absolute presence of either of these functional groups, taken together they do suggest a microeukaryotic presence in these sediments and that the presence was greater in the coastal plain than in the arid west.

In summary, a range of sediments recovered from the deep subsurface showed viable microbial biomass levels on the order of one magnitude less than those observed in near surface sediments. The diversity of PLFA detected decreased as well, due in part to the low biomasses. However, differences in microbial community structure between deep subsurface communities could be identified and related to differences in fatty acid biosynthesis and nutritional/physiological status.

8.3.2.2.1 Relationships of Microbial Community Structure and Geochemical Gradients

Another example for the utility of the SLB analysis is in discerning the *in situ* microbial ecology along geochemical gradients. One example is the research performed in

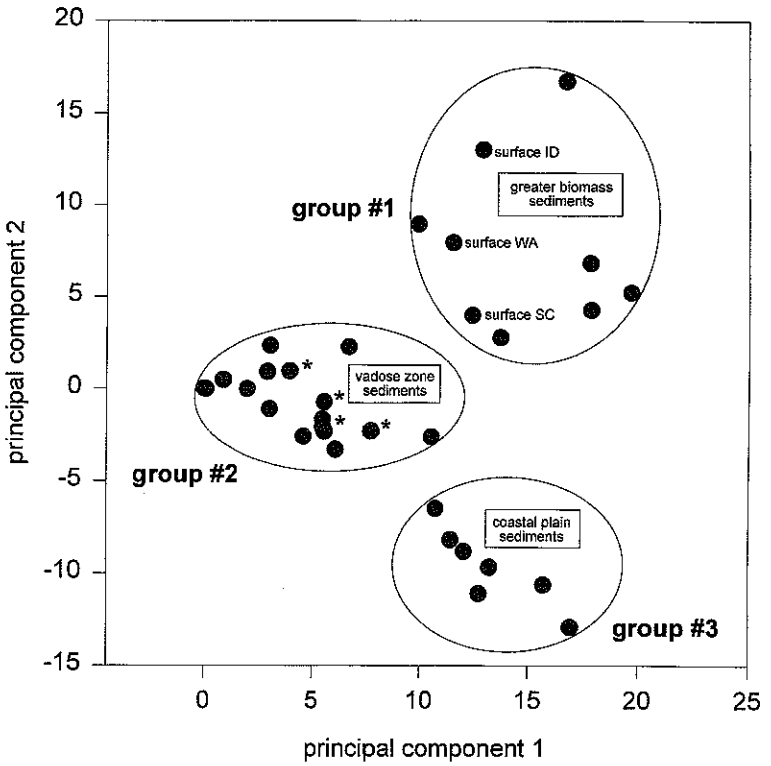

FIGURE 8.5
A 2-D representation of a principal components analysis of PLFA mole % profiles obtained from deep subsurface sediments recovered from sites in SC, ID, and WA. Group #1 contains sediments exhibiting a viable biomass >8 pmol g^{-1} including the surface soils. Group #2 contains almost all of the vadose zone samples from the two western sites as well as those sediments exhibiting a high (>0.1) *trans/cis* ratio (indicated by an *). Group #3 contains the subsurface coastal plain sediments.

support of the Geological Microbiological Hydrogeological Experiment (GEMHEX) conducted at the Hanford site near Richland, WA (Fredrickson et al., 1995). Deep subsurface sediment samples were recovered over a depth interval of 174 to 195 m that included three distinct geological zones: a lacustrine, a paleosol, and a fluvial sand. Viable microbial biomass estimates peaked in the lacustrine sediments, reaching a maximum of 45 pmol PLFA per gram after starting at background levels of <1 pmol PLFA per gram. Values were observed to gradually decrease through the paleosol, reaching background levels by the end of this zone and throughout the fluvial sands. The peak in viable microbial biomass was characterized by an increase in the proportions of both terminally branched saturated and monounsaturated PLFA. A more detailed analysis of the terminally branched saturated PLFA indicated that the ratio of *iso/anteiso* heptadecanoic acid (17:0) was greater than twice that observed in the adjacent sediments. Ratios of i17:0/a17:0, at the levels observed in the lacustrine sediments, were found to be characteristic of certain *Desulfovibrio* species of sulfate-reducing bacteria. Corroborative evidence for the presence of sulfate-reducing bacterial populations was found through the positive hybridization of recovered DNA with a 16S rRNA probe specific for sulfate-reducing bacteria in the lacustrine sediments. The lacustrine sediments exhibited a very low hydraulic conductivity (10^{-7} to $>10^{-9}$ cm s^{-1}), a total organic content of ~1%, a higher carbonate content, and lower pore water sulfate than the surrounding sediments, all of which suggest *in situ*

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