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FEMS Microbiology Reviews 20 (1997) 371–377

FEMS MICROBIOLOGY REVIEWS

Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipid fatty acids

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Abstract

To understand the ecology of microorganisms in deep subsurface sediments, it is necessary to utilize analyses which look at communities in situ since culturable organisms represent a small fraction of the extant microbiota. Application of the signature lipid biomarker (SLB) technology provides quantitative measures of viable biomass (abundance and distribution), community composition and insight into the nutritional physiological status of the community. With this analysis it was possible to define differences in the communities of different geologic horizons with differing geochemical and geophysical compositions. Although a number of different sites across the continental United States have been studied, the focus of this report is on a single site located in the semi-arid west in New Mexico state where late cretaceous (120–135 Myr) shales and sandstones could be sampled. Sediment cores from this site yielded ester-linked phospholipid fatty acids (PLFA) indicating the presence of viable microbiota as well as diglyceride fatty acids (DGFA) which described the recently dead or non-viable populations of microorganisms. Both the spatial distribution and the nature of PLFA and DGFA were examined under the premises of two hypotheses: (1) that microorganisms in unheated shales would be similar to modern near shore marine sediment organisms and would be absent from heated (volcanic) shales and (2) that different microbial communities would be detected in shales and sandstones as a result of environmental influences and restrictions both at the time of deposition and in subsequent diagenesis.

Keywords: Microbial ecology; Ester-linked phospholipid fatty acid; Signature lipid biomarkers; In situ

Contents

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PII S0168-6445(97)00019-3

1. Introduction

The analysis of deep subsurface microbiota requires first and foremost that sample material be acquired in a manner which minimizes the potential for contamination. To monitor the potential for contamination with the recovery of sedimentary samples collected from 10 000 feet, artificial tracers were incorporated into the drilling technology. The tracers proved to be a successful means of assuring quality control [1]. Another successful means by which sample quality could be assured was through the analysis of inherent tracers such as ester-linked phospholipid fatty acids (PLFA) and community level bioassays [2].

Once samples of sufficient quality were obtained, the task then was in the quantification and identification of the extant microbiota. Classical techniques which attempted to isolate and culture deep subsurface organisms often resulted in the description of less than 10% of the biota present. These techniques not only failed to describe the total community, they obscured possible interactions and quantitative estimates of heterogeneity in the distribution of microbes and microbial activities. Consequently, in situ techniques which identified traits of the community as a whole, such as the signature lipid biomarker (SLB) technique, were applied. Application of the SLB technology to deep subsurface environments has resulted in insight into the distribution, abundance and relationships of viable microbial communities to relation to differing geophysical and geochemical conditions [3,4]. The analyses reported herein describe SLB results from sediment cores recovered from a series of alternating late cretaceous shales and sandstones.

The Department of Energy Subsurface Science program under the direction of Dr. F.J. Wobber drilled a borehole near the town of Cerro Negro in New Mexico, USA. A more complete description of the site, the drilling techniques and quality assurance measures employed can be obtained from Griffin [5]. A detailed description of the geology and geochemistry of the site is given in Fredrickson et al. [6]. The borehole was drilled to test two hypotheses: (1) a thermal aureole hypotheses which proposed that microorganisms in unheated shales would be similar in nature to those found in modern near shore marine sediments and that microorganisms would be absent from heated (volcanic) shales and (2) a shale sandstone hypothesis which proposed that different microbial communities would be detected in shales compared to sandstones as a result of the differing influences and restrictions these two substratum would have placed on the extant microbiota [5]. In order to address these two hypotheses, PLFA and DGFA were quantitatively extracted from the sediments, isolated and identified providing insight into the abundance and community composition of the viable and non-viable extant microbiota.

2. Materials and methods

Samples were obtained from two separate boreholes, one vertical and one angled. The vertical borehole did not penetrate the thermal impact zone whereas the angled borehole allowed sampling of heated sediments. Details of the drilling process can be obtained from Griffin [5]. Sediment cores from each borehole were recovered aseptically as described in the work by Colwell et al. [1]. Following collection and processing, samples were shipped via overnight mail to the Center for Environmental Biotechnology at the University of Tennessee (Knoxville, TN, USA).

In our laboratory, core material collected from different depth intervals was pulverized using sterile utensils and extracted in a single phase chloroform:methanol:phosphate buffer (1:2:0.8, v:v:v) solution [7]. After 2 min of sonication the mixtures were allowed to extract for a period of 3 h. Following this period, additional aliquots of chloroform and water (1:1, v:v) were added to the mixture resulting in a two phase system. The organic phase was collected, dried and subjected to fractionation on silicic acid columns using sequential eluents of chloroform, acetone and methanol (5 ml each) [8]. Diglycerides were then isolated from the chloroform fraction by thin layer chromatography [9]. Fatty acids of the phospholipids (from the methanol fraction) and the isolated diglycerides were then transesterified into methyl esters using methanolic potassium hydroxide [8]. The individual fatty acids were further separated, quantified and identified using capillary gas chroma-

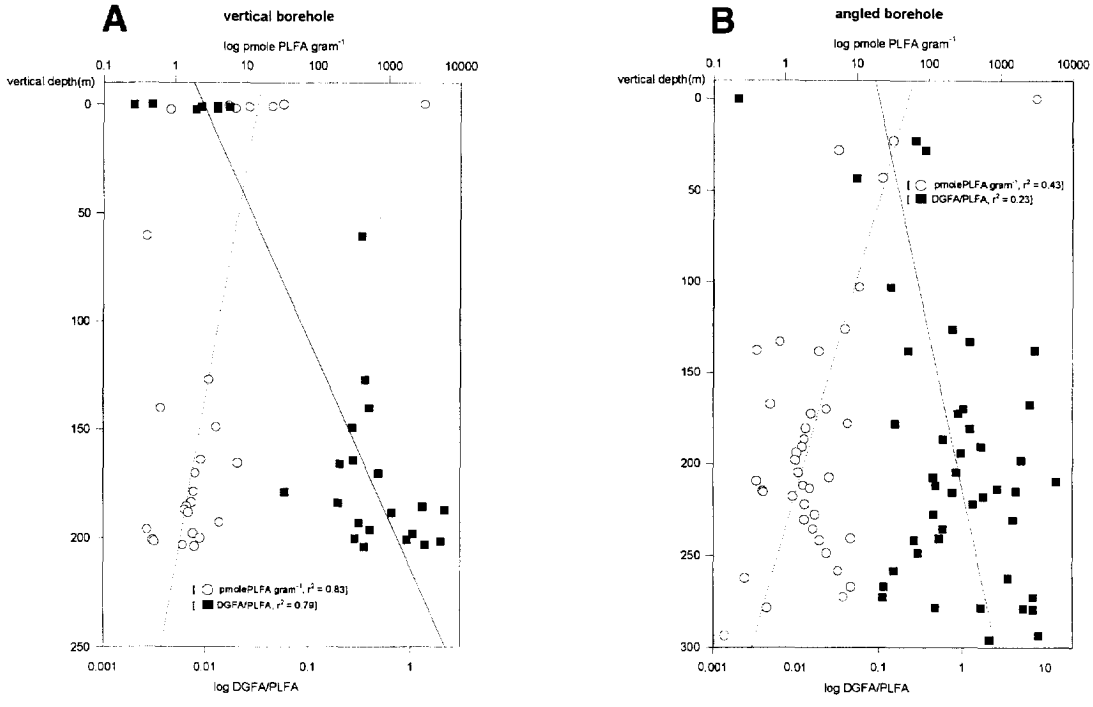

Fig. 1. Plots of PLFA abundance (log pmol g^{-1}) versus depth (circles) and DGFA/PLFA (log) versus depth (squares) observed in sediment cores recovered (A) throughout the vertical borehole and (B) in sediment cores throughout the angled borehole at a site near Cerro Negro, NM. Linear correlations (expressed as R^2 values) for each plot are indicated.

tography coupled to a mass spectrometer operating in the positive ion electron impact mode [9].

Results were analyzed using correlation statistics, analysis of variance (ANOVA) and hierarchical cluster analysis. The three tests were utilized to ascertain whether correlations existed between viable and nonviable cell biomass, to determine significant difference (Tukey HSD) between means of individual and summed PLFA molar percentages and to determine relatedness between PLFA profiles. All statistical analyses were performed using Statgraphics Plus for Windows 95 (Rockville, MD, USA).

3. Results and discussion

Within the vertical borehole (Fig. 1A) significant correlations were observed between core depth and the log of the PLFA abundance ($R^2 = 0.83$) as well as core depth and the log of the ratio of DGFA/PLFA ($R^2 = 0.79$). Within the angled borehole, however, a decrease in the significance of these two correlations was observed. PLFA versus depth showed an R^2 of 0.42 and the ratio of DGFA/PLFA an R^2 of only 0.23 (Fig. 1B). The decreased significance in the biomass correlations observed in the angled borehole may be attributed to the hypothesized thermal impact. These sediments represented Paquate sandstone (to 170 m), Clay Mesa shale (to 214 m), Cubero sandstone (to 262 m) and the Morrison formation stratigraphic units [6].

The greatest depths attained by the angled borehole reached the Morrison formation which geological evidence indicated was subjected to volcanic heating [5]. The heat encountered during the volcanic stage would have likely eliminated all microbial cells and cell components (PLFA and DGFA). Fig. 2 illustrates that a lack of PLFA abundance was encountered within this formation as compared to the three geological horizons immediately above. Of the nine cores collected throughout the Morrison horizon, two showed PLFA abundance above background levels (both located near the top of the horizon) and no cores showed DGFA abundance above

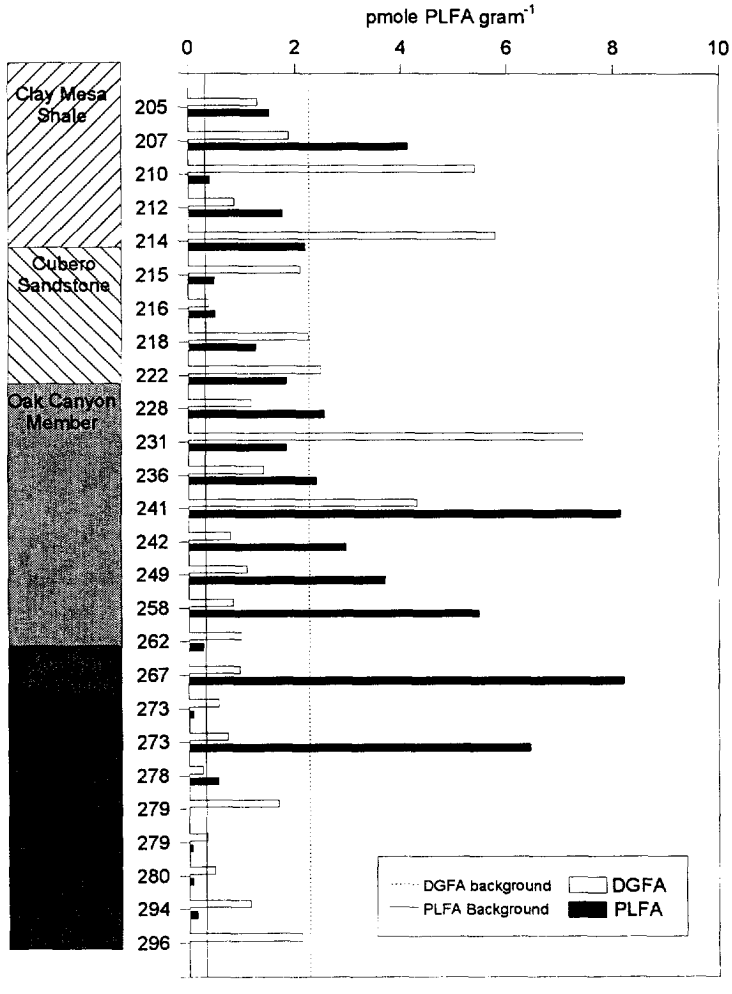

Fig. 2. Viable (PLFA) and non-viable (DGFA) microbial biomass estimates observed throughout the lower portion of the angled borehole. The solid line indicates the background level of PLFA detected in procedural controls and the dashed line the background for the DGFA assay. The geologic horizons penetrated by the borehole are indicated in the box to the left of the plot.

background. These results are supportive of the hypothesis that past volcanic heating adversely effected the extant microbial populations and their PLFA and DGFA signatures.

Three sandstone and three shale horizons were encountered throughout the angled borehole. The mean mole percentages for the prominent PLFA detected in each are given in Table 1. An arcsin transformation of the mole percentages were analyzed by application of a K-means clustering algorithm. The method successfully placed 71% (15 of 21) of the PLFA profiles obtained from shale and sandstone cores in their respective groups (i.e., sandstone or shale). Of the six core PLFA profiles incorrectly placed, three were samples located at a transition point between a sandstone and a shale horizon. The other three cores showed no unique features and their placement in cluster groups contrary to their lithological characteristics is likely a result of some other environmental restriction. These cores were removed from the data set prior to the development of Table 1.

In Fig. 3, differences in the PLFA profiles obtained from the Mancos shale and Two Wells sand-

Table 1
Molar percentages of PLFA recovered from different geologic horizons (averaged within a horizon) throughout the angled borehole

Lithology	Mancos shale		Two Wells sandstone		Whitewater Arroyo shale	Paquate sandstone		Clay Mesa shale	Cubero sandstone	
Depth (m)	43–126		133–138		173	187–198		212	214–222	
	mean	S.D.	mean	S.D.		mean	S.D.		mean	S.D.
Ester-linked phospholipid fatty acids										
n14:0	8.57	3.73	6.51	5.14	4.27	8.08	2.19	8.85	9.61	5.19
n15:0	9.41	4.24	6.92	5.00	3.84	6.19	4.57	5.74	4.78	5.97
n16:0	21.07	4.81	37.41	1.62	39.04	27.88	1.82	24.24	25.09	2.14
n17:0	7.36	1.84	4.64	3.28	7.45	11.51	2.22	10.26	16.07	10.06
n18:0	6.56	2.16	22.84	0.71	18.59	23.01	2.56	17.04	29.40	13.25
i15:0	2.65	1.01	1.27	1.80	1.56	1.13	0.76	0.00	0.77	0.81
a15:0	2.14	0.74	1.01	1.43	1.64	1.80	1.04	0.00	0.81	0.80
i16:0	2.80	1.06	0.00	0.00	3.26	2.09	1.36	0.00	0.15	0.22
i17:0	4.89	4.08	0.00	0.00	0.00	0.67	1.17	0.00	1.11	1.57
a17:0	2.20	1.87	0.00	0.00	3.12	2.07	2.10	0.00	0.32	0.45
i17:1w7c	4.10	2.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1w7c	5.17	0.30	0.00	0.00	2.87	2.28	2.28	0.00	0.44	0.62
cy17:0	4.63	3.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18:1w7c	4.40	3.71	0.00	0.00	4.74	0.00	0.00	10.39	0.00	0.00
Functional group totals										
normal saturates	60.82	10.37	97.72	3.23	82.80	89.67	6.92	89.61	95.85	3.61
terminally branched saturates	16.54	2.21	2.28	3.23	9.59	8.05	5.02	0.00	3.72	3.72
monounsaturates	22.64	10.51	0.00	0.00	7.61	2.28	2.28	10.39	0.44	0.62
	$n=3$		$n=3$		$n=1$	$n=4$		$n=1$	$n=3$	

stone horizons are illustrated. The sandstone showed no detectable levels of the terminally branched saturates i16:0, i17:0 and a17:0 or the monounsaturates i17:1w7c, 16:1w7c, cy17:0 or 18:1w7c, while their presence was readily detected in the shale horizon. The presence of *iso* terminally branched saturates and the terminally branched monounsaturate i17:1w7c is typically associated with *Desulfovibrio* sp. [10]. In general, shales throughout the angled borehole showed greater percentages of this and other monounsaturated PLFA. Monounsaturated PLFA are typically found in Gram-negative cell membranes [11] but include organisms such as the sulfate reducing bacteria just mentioned [10].

The sandstones showed significantly greater percentages of the normal saturates 16:0 and 18:0 which are prominent PLFA in most all viable cell membranes, prokaryotic and eukaryotic [12]. In general, the sandstones showed greater percentages of all of the normal saturates detected (Table 1). An increase in the saturation of a cell membrane can reflect a decrease in membrane fluidity [13] suggesting that this observation may be related to cell physiology as well as community composition.

4. Conclusions

The documentation of a loss of PLFA within the Morrison formation of the angled borehole suggests that an event occurred in the past which eliminated a viable microbial presence. This was further substantiated by the absence (less than background levels) of DGFA in the same horizon, although substantial levels of DGFA and detectable levels of PLFA were observed in the horizon just above. The data reported here supports the thermal impact hypothesis but further research into the ability of a subsurface organism to withstand high temperatures under conditions of desiccation is needed to estimate temperature and exposure times necessary for the destruction of the extant microbiota.

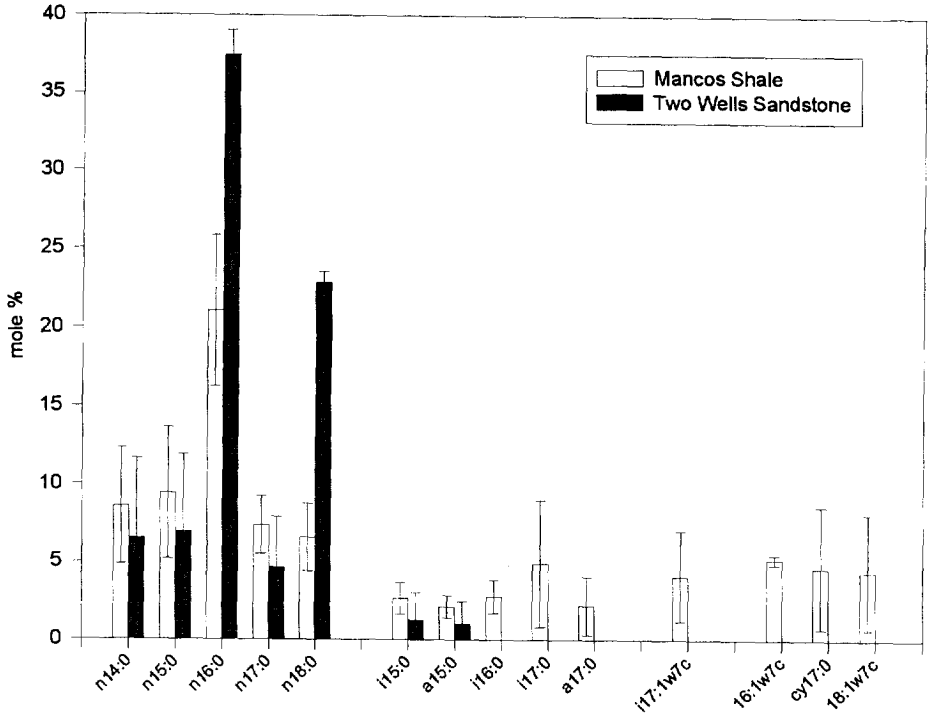

Fig. 3. Plot illustrating differences in PLFA profiles observed between sediment cores collected from the Mancos shale horizon and the Two Wells sandstone horizon. Error bars indicate one standard deviation.

The differences in PLFA patterns described above suggest that different microbial communities exist in deep subsurface sandstone and clay horizons. This observation is in support of the hypothesis that environmental restrictions on microbial growth encountered in these two different geologic horizons select for different microorganisms. The observation of PLFA indicative of the sulfate reducing bacteria, *Desulfovibrio*, in the shales is consistent with what is observed in near shore benthic marine sediments. This raises the possibility that deep subsurface microbiota may have survived in these sediments since the time of deposition.

Although the analysis of the extant microbial communities by SLB support the two hypotheses and the analysis of PLFA abundance and patterns provides insight into the ecology of deep subsurface microbiota not obtainable with classical microbiological techniques, the SLB analysis alone is not sufficiently specific to prove or disprove such hypotheses. One drawback of the analysis is a lack of specificity in the identification of individuals within the community. Coupling the SLB analysis with the analysis of nucleic acids can improve the comprehensiveness of the two in situ measures. An integrated approach which utilizes both technologies will provide a comprehensive and accurate description of deep subsurface microbial communities possibly allowing for a more definitive answer to the proposition that some of the differences observed here with these microbial communities are the result of differences in the conditions at the time of deposition. Insight into how community composition could then be further modified by incorporation of groundwater organisms and through the subsequent diagenic history may then become attainable.

Acknowledgments

This research was supported by a grant (DE-FG02-96ER62163) from the Department of Energy Office of Health and Environmental Resources Subsurface Science Program administered by Dr. Frank

Wobber. We thank the researchers and drillers who aided in the collection of the samples and the personnel at the Center for Environmental Biotechnology for help in sample processing.

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