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Association of acid-producing thiobacilli with degradation of concrete: analysis by 'signature' fatty acids from the polar lipids and lipopolysaccharide

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SUMMARY

The acid-producing thiobacilli contain fatty acid components in the polar lipids and lipopolysaccharide lipid A that are sufficiently unusual that they can be utilized as 'signature' lipid biomarkers for these organisms in environmental samples. Studies in microcosms have shown correlations between activity of these organisms, measured by recovery and viable counting, and the degradation of concrete. The 'signature' lipid analysis provides a detection assay requiring neither separation of the organisms from the substratum nor growth prior to determination. The presence of acid-producing thiobacilli was demonstrated in microcosm samples and degenerating concrete from the Hamburg (F.R.G.) sewer system.

INTRODUCTION

Signature lipid biomarker fatty acids of the polar lipids (PLFA) have been utilized to define the biomass and community structure of microbial consortia in biofilms, soils and sediments [20–24]. The total PLFA is an excellent measure of microbial biomass, and the presence of specific patterns of PLFA that are restricted to specific subsets of the total microbial community can be utilized to define the community structure. Signature PLFA biomarkers have been utilized to detect type I and II methanotrophic bacteria [13,15], *Desulfobacter* and *Desulfovibrio* sulfate-reducing bacteria [1,2], the pathogenic bacterium *Francisella tularensis* [14], and the archaebacterial methanogenic bacteria [9,10].

The acid-producing, aerobic, gram-negative thiobacilli have been shown to produce a diverse complement of fatty acids in the chloroform:me-

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thanol-extractable phospholipids [3,5,7]. The combination of *o*-methoxy, mid-chain hydroxy, 2-hydroxycyclopropane, midchain-branched, branched-cyclopropane, monounsaturated at the omega (ω) 5,6,7,8,9,10 positions, 2-hydroxy and 3-hydroxy, and large proportions of the 'thiobacillic' cyclopropane 19:0 ω (8,9) fatty acids are found in the polar lipids of these organisms. These PLFA are unique amongst the microbes, so that they can serve as effective 'signatures' for these organisms.

Failures of concrete sewers have been associated with acid-producing microorganisms that utilize reduced sulfur and oxygen with the generation of sulfuric acid [11,16,19,21]. To study the resistance of different concrete samples to the effects of the thiobacilli, Sand and Bock constructed a chamber in which the temperature, humidity, and hydrogen sulfide concentration could be regulated [18,19], and were able to greatly accelerate the biodegradation. In the field and in the artificial chamber, the most rapid biodegradation was associated with the growth of *Thiobacillus thiooxidans* [11,18]. The detection of the thiobacilli in these studies involves isolation and culture on several media. This can require 6–8 weeks. The present study shows that the presence of the acid producing thiobacilli can be detected in samples from the Hamburg sewer and from the biotest chamber, utilizing the 'signature' biomarkers from the extractible PLFA, and the lipopolysaccharide lipid A (LPS).

MATERIALS AND METHODS

Materials

Solvents and reagents were analytical grade or better. Standards and derivatizing agents were purchased from Supelco, Inc. (Bellefonte, PA), Applied Science (State College, PA), Aldrich, Inc. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), and Pierce Chemical Co. (Rockford, IL).

Samples

Thiobacillus thiooxidans strain K-2, *T. neapolitanus* strain B-2 and *T. intermedius* strain D-14 were grown and then recovered in the late stationary phase by centrifugation as described previously [5,18]. These strains were isolated from the sewer network of Hamburg. They have been characterized [10]. Concrete samples of 50–400 g from the sewer or biotest chamber were lyophilized and then pulverized to chunks smaller than 1 cm^3. Concrete test samples had been corroded to a depth of several millimeters. The samples from the biotest system lost an average of 3.3% of their weight.

Extraction

The modified one-phase Bligh and Dyer extraction was utilized for all samples [24]. Duplicate samples were extracted separately, and all data are expressed as the mean of two determinations. After overnight separation of the lipid and aqueous phases in the second stage of the extraction, the organic fraction was filtered through fluted Whatman 2V filters and evaporated to dryness under a stream of nitrogen. To recover the polar lipids, silicic acid columns were prepared using 1 g Unisil (100–200 mesh) (Clarkson Chemical Co., Inc., Williamsport, PA) activated at 120°C for 60 min and pre-extracted with chloroform. The columns were 14-mm-diameter glass columns. Total lipid was applied to the top of the columns in a minimal volume of chloroform. The column was eluted with 10 ml of chloroform and the neutral lipids were recovered. This was followed by 10 ml of acetone from which the glycolipids were recovered. The column was eluted with 10 ml of chloroform and the polar lipids were recovered. The polar lipid fraction was dried under a stream of nitrogen. The mild alkaline methanolysis procedure [24] was utilized to prepare methyl esters of the ester-linked PLFA.

Lipopolysaccharide analysis

The residue from the lipid extraction of the cells was refluxed in 3 N HCl for 4 h and the lipid fraction was recovered in chloroform [17]. Concrete samples were refluxed in 5% (w/v) KOH in methanol:water (8:2) for 4 h, allowed to cool, neutralized to pH 6.0, and the hydrolysate was separated from the concrete by repeated washes with distilled water and chloroform. The solution was partitioned against chloroform, and the lipid-soluble hydrolysate was recovered.

Purification of the lipids

Thin layer chromatography (TLC) on Whatman K-6 silica gel, 0.250 mm thick, was utilized for purification of lipid fractions. The methyl esters from the polar lipid fraction were applied in a strip to the origin of the TLC plate that had previously been divided into a large mid-plate channel with two narrow channels on the edges. Authentic fatty acid methyl esters (FAME) and hydroxy fatty acid methylesters (OHFAME) were applied to the outside channels, and the plate was placed in a tank for separation by ascending chromatography in a solvent of chloroform:methanol:water (55:35:6, v/v). The silica gel bands corresponding to the FAME and OHFAME ($R_F > 0.8$) were lifted from the plate with vacuum in a Pasteur pipette, and the fatty acids were recovered with chloroform:methanol (2:1, v/v). The position of the aminolipids was identified by spraying a portion of the plate with 0.25% w/v ninhydrin in acetone:lutidine (9:1, v/v) [4]. The bands on the unsprayed portion of the plate were recovered in a Pasteur pipette and eluted with chloroform:methanol (1:1 and 2:1, v/v).

The FAME and OHFAME were separated using ascending chromatography with hexane:diethyl ether (1:1, v/v). The FAME band (R_F 0.65) and OHFAME band (R_F 0.25) were recovered and eluted with chloroform and chloroform:methanol (1:1, v/v), respectively. The 2-OH and 3-OHFAME were not separated with the GC conditions reported here. The presence of each was confirmed by GC/MS.

Aminolipids recovered from the TLC plates were subjected to acid methanolysis in anhydrous methanol:concentrated HCl:chloroform (10:1:1, v/v) and incubated at 100°C for 1 h. The OHFAME were recovered in chloroform.

Derivatizations

Trimethylsilyl ethers of OHFAME were formed with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSFTA) (Pierce Chemical Co., Rockford, IL) [17].

The position and geometry of the monounsaturation in the FAME and OHFAME were determined using two procedures. Dimethyl disulfide (DMDS) adducts were prepared as described [12]. These derivatives increase the resolution between *cis* and *trans* geometrical isomers in capillary gas-liquid chromatography (GC). The position of the cyclopropane ring in the FAME can be determined after hydrogenation in the presence of Adam's catalyst of PtO_2, with the esters dissolved in methanol:glacial acetic acid (1:1, v/v) under a hydrogen atmosphere (140 kPa) at room temperature with mechanical agitation for 20–40 h in a Parr hydrogenation apparatus (Moline, IL) [5]. These derivatives form a mixture of straight-chain and branched FAME with branch points on either side of the original ring position. The mass fragments of the two branched FAME give the location of the two branches and thus the original ring position. Similar treatment of the 2-OH-cyclopropyl FAME did not yield fragments allowing determination of the branch points. The samples recovered in these experiments were insufficient to allow quantitative determination of the proportions of the 2-hydroxy or 3-hydroxy fatty acids. Fragmentation patterns indicated that both were present in these samples.

Gas chromatography (GC)

Dry FAME or OHFAME were dissolved in hexane, and the internal standard of methyl nonadecanoate was added. Samples (1.0 μl) were injected onto a 50-m nonpolar, cross-linked methyl silicone fused silica capillary column (0.2 mm i.d., Hewlett Packard) in a Varian 3700 GC. A 30-s splitless injection with the injection temperature at 250°C was used. Hydrogen at a linear velocity of 35 cm/s was the carrier gas, with a temperature program starting with an initial temperature of 80°C. An initial 20°C/min rise for 3 min followed by a 4°C/min rise for 30 min and an isothermal period for the remainder of the 40-min program was utilized. Detection was by hydrogen flame (FID) using a 30 ml/min nitrogen make up gas at a temperature of 290°C. An equal detector response was assumed for all components. Peak areas were quantified with a programmable laboratory data system (Hewlett Packard 3350 series) operated in an internal standard program relative to known amounts of internal standard.

Gas chromatography/mass spectrometry (GC/MS)

FAME and OHFAME were tentatively identified by co-elution with authentic standards supplied by Supelco, Inc. (Bellefonte, PA) and Applied Science Labs., Inc. (State College, PA) or previously identified laboratory standards. The analysis was performed on a Hewlett Packard 5996A GC/MS with a direct capillary inlet, utilizing the same chromatographic system except for the temperature program, which was begun at 100°C and increased to 280°C at 4°C/min for a total analysis time of 60 min. The electron multiplier voltage was between 1400 and 1600 V, the transfer line maintained at 300°C, the source and analyzer maintained at 250°C, and the GC/MS was autotuned with DFTPP (decafluorotriphenylphosphine) at m/z 502 with an ionization energy of 70 eV. The data were acquired and processed using the Hewlett Packard RTE 6/VM data system. Other conditions were as described previously [5].

Fatty acid nomenclature

Fatty acids are designated as total number of carbon atoms:number of double bonds, with the position of the double bond nearest to the aliphatic (ω) end of the molecule indicated. This is followed by the suffix c for *cis* and t for *trans* configuration of monoenoic fatty acids. The prefixes i, a, or br indicate iso, anteiso, or branched (position undetermined). Mid-chain branching is indicated by the number of carbon atoms from the carboxyl ( ) end of the molecule and Me for the methyl group. Cyclopropane rings are indicated with the prefix cy and the position of the ring from the aliphatic (ω) end of the molecule. Hydroxy fatty acids are indicated by the number of carbon atoms from the carboxyl end of the molecule followed by the prefix OH. Methoxy fatty acids are given with the number of carbon atoms from the carboxyl end of the molecule followed by the prefix MeO.

RESULTS

Detection of thiobacilli

The proportions of ester-linked FAME and both ester- and amide-linked OHFAME in the polar lipids in the thiobacilli isolated from corroding concrete, as test concrete block exposed in a biotest chamber to thiobacilli, and a sample from a corroding concrete sewer pipe are listed in Table 1A. The OHFAME from the LPS-lipid A of the lipid-extracted residue of the isolated monocultures and the test and environmental samples are given in Table 1B. The similarity in the patterns of proportions of FAME and OHFAME from the monocultures, biotest samples, and the corroded sewer sample indicate that the acid-producing thiobacilli are a prominent part of the microflora in both the biotest and sewer samples. The low proportions of the usual bacterial PLFA such as 16:0, 18:1ω9c, cy17:0ω(7,8), the elevated proportions of the unusual 'thiobacillic' acid (cy19:0ω(7,8) in the extractable polar lipid FAME, the 2-OH and 3-OH16:0 in the ornithine-lipid, and the high proportion of OHFAME in the PLFA as well as the high proportion of 2-OH and 3-OH16:0 in the LPS-lipid A are typical of the acid-producing thiobacilli. Hydroxy-cyclopropyl fatty acids found in both the extractable polar lipids and the LPS-lipid A are typical of the acid-producing thiobacilli [5].

Detection of T. thiooxidans

The elevated proportions of 2-OH14:0, 2-OH16:0, and particularly 2-OHcy19:0, and the high OHFAME/FAME ratio in the extractable polar lipids of the biotest chamber and environmental samples, together with the high proportions of 3-OH14:0 and 2-OH and 3-OH19:0, suggest that *T. thiooxidans* is a predominant member of the microbial consortia in the environmental samples. The higher proportion of 3-OH12:0 in the LPS of both the biotest sample and the environmental samples more closely parallels the pattern in *T. intermedius* and *T. neapolitanus* than that in the *T. thiooxidans* monoculture. This suggests that the other thiobacilli are also part of the community.

DISCUSSION

Signature PLFA

It has been possible to utilize the 'signature' lipid biomarker technique to detect specific microorgan-

Table 1

(A) Ester-linked fatty acid composition in the polar lipid of thiobacilli

Values given are mol% of total FAME or OHFAME, and represent average values of duplicate analyses. Nomenclature of fatty acids is defined in Materials and Methods.

	T. intermedius	*T. neapolitanus*	*T. thiooxidans*	Chamber	Sewer
Nonhydroxy fatty acid					
15:0	—	3.79	—	1.36	6.84
16:1ω7C + T	0.87	15.1	9.19	3.25	8.54
16:0	15.0	28.5	36.1	15.8	23.5
cy17:0ω(7,8)	9.46	1.20	5.56	10.8	2.43
17:0	2.64	4.61	< 0.3	2.46	14.5
18:1ω7C + T	6.63	37.2	39.5	18.8	15.9
18:1ω5C	0.95	0.93	< 0.3	< 1.0	< 1.0
10 + 11Me18:1ω6	5.97	0.41	0.70	< 1.0	< 1.0
cy19:0(ω8,9)	25.6	1.52	4.24	32.8	14.7
cy20:0	11.6	0.34	1.35	< 1.0	< 1.0
unidentified	21.1	6.4	< 0.1	13.5	11.8
Amide and ester-linked hydroxy fatty acid					
2-OH and 3-OH14:0	0.59	5.95	5.74[a]	11.8[a]	21.5[a]
2-OH-cy16:0	6.44	28.2	—	5.99	10.7
2-OH and 3-OH16:0	53.6[a]	10.9[a]	25.0[a]	16.5[a]	11.1[a]
2-OH and 3-OH17:0	1.28	3.32	2.92	0.96	1.73
2-OH-cy18:0	11.0	38.0	1.61	3.70	1.11
2-OH and 3-OH18:0	5.27	5.72[a]	22.6	10.3	15.2
2-OH-cy19:0	15.3	8.02	42.2	27.3	6.69
unidentified	19.4	< 0.1	< 0.1	23.4	31.9
% OHFAME/FAME	12.3	1.5	15.3	33.1	42.4

[a] Indicates found primarily in the polar aminolipid.

(B) Hydroxy fatty acid composition of the lipopolysaccharide of extracted thiobacilli

	T. intermedius	*T. neapolitanus*	*T. thiooxidans*	Chamber	Sewer
Hydroxy fatty acid					
2-OH and 3-OH12:0	39.1	57.1	1.41	19.5	14.5
2-OH and 3-OH13:0	1.98	8.94	4.37	< 1.0	< 1.0
2-OH and 3-OH14:0	6.48	5.81	20.6	26.4	21.4
2-OH and 3-OH15:0	< 1.0	8.57	4.31	5.48	7.27
2-OH and 3-OH16:0	44.2	7.93	32.6	13.3	11.6
2-OH and 3-OH17:0	< 1.0	< 1.0	< 1.0	1.28	2.82
2-OH and 3-OH18:0	8.27	4.15	9.18	5.67	11.1
2-OH and 3-OH19:0	< 1.0	3.41	9.82	9.62	5.42
unidentified	< 0.1	3.6	17.1	18.1	25.3

isms in complex environmental samples. Methane-oxidizing auxotrophs contain PLFA that are sufficiently unusual that they can be utilized as biomarkers [15]. Exposure of columns of sub-surface soil to natural gas resulted in a great increase in the specific PLFA of type II methylotrophs [13]. The methaneogenic Archaebacteria contain bi- and bi-diphytanyl glycerol ether polar lipids which can be assayed in environmental samples and correlated with methanogenic activity in soils and sediments

[9,10]. The pathogenic bacteria *Francisella tularensis* and the sulfate-reducing bacterial groups of lactate-utilizing *Desulfovibrio* and acetate-utilizing *Desulfobacter* contain sufficiently unusual patterns of PLFA to allow their assessment in mixed microbial assemblies [1,2,12]. The diversity of unusual FAME and OHFAME in the acid-producing thiobacilli allows their detection and even allows some differentiation between species in environmental samples (Table 1). Better purification of the lipids with larger samples would allow separation of the 2-OH and 3-OHFAME in the polar lipid and LPS preparations. This would increase the discriminatory power of the analysis to species differences. With the samples used in this paper, the proportions of 2-OH and 3-OHFAME were estimated from the GC/MS fragmentation patterns.

With the FAME and OHFAME patterns of the thiobacilli available (Refs. 3, 5 and 8; Table 1), it becomes possible to study their interactions with other microorganisms whose PLFA patterns are also known [21–23]. These known patterns can be factored into analyses of environmental samples subjected to various perturbations, and the effects on the community structure can be determined. Using this technique, detrital succession, the effects of disturbance or predation upon marine sediments, the response to subsurface aquifer microbiota to pollution, and the effects of shifts in the microbial biofouling succession and facilitation of corrosion have been documented [20,22,23].

Metabolic status

Many bacteria accumulate cyclopropane fatty acids as the community ages or undergoes nutritional stress [6]. Their formation, with the concomitant decrease in monoenoic PLFA, occurs in monocultures that undergo metabolic stress such as stationary phase growth [4,6]. This same phenomenon has been detected in the benthic marine microbiota [4,23] as well as the acid-producing thiobacilli [5]. The acid-producing thiobacilli from the chamber samples and from the degenerating concrete sewers both show the high levels of cyclopropane fatty acids and low levels of monoenoic PLFA characteristic of late stationary growth phase.

Role of acid-producing thiobacilli in biodegradations

The correlation between the degree of biodeterioration of concrete and the activity of acid-producing thiobacilli has been shown using classical recovery and culture techniques [11,18,19]. The studies reported in this paper indicate that the PLFA patterns are sufficiently specific to define the presence of the acid-producing thiobacilli and possibly to define the particular species that are present without the uncertainties and time delays of cultural methods. In addition, the interpretation of PLFA profiles offers insight into the community structure and metabolic status of the total microbial community associated with biodegradation of materials that involve these organisms. Preliminary evidence from both corroding sewer systems and a continuous culture apparatus, designed to test the resistance of concrete samples to the corrosive activities of acid-producing bacteria, shows that the degree of biodegradation appears to correlate with the presence of 'signature' PLFA of the acid-producing thiobacilli, particularly *T. thiooxidans* [11,18,19]. The methodology described herein will allow examination of an entire microbial ecosystem, so that the interactions between the acid-producing thiobacilli and other organisms that potentiate their corrosive activities can be defined [8].

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REFERENCES

1 Dowling, N.J.E., F. Widdel and D.C. White. 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulfate reducers and other sulfide forming bacteria. J. Gen. Microbiol. 132: 1815–1825.

2 Edlund, A., P.D. Nichols, R. Roffey and D.C. White. 1985.

Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. J. Lipid Res. 26: 982–988.

3 Ghosh, M. and A.K. Mishra. 1985. Effect of cultural conditions on the lipid profile of *Thiobacillus ferrooxidans*. Arch. Microbiol. 143: 297–301.

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

5 Kerger, B.D., P.D. Nichols, C.A. Antworth, W. Sand, E. Bock, J.C. Cox, T.A. Langworthy and D.C. White. 1986. Signature fatty acids in the polar lipids of acid producing thiobacillus spp.: methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl and branched and normal monoenoic fatty acids. FEMS Microbiol. Ecol. 38: 67–77.

6 Knivett, V.A. and J. Cullen. 1965. Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem. J. 96: 771–776.

7 Knoche, H.W. and J.M. Shively. 1972. The structure of an ornithine-containing lipid of *Thiobacillus novellus*. J. Biol. Chem. 247: 170–178.

8 Kuenen, J.G., L.A. Robertson and H. van Gemerden. 1985. Microbial interactions among aerobic and anaerobic sulfur-oxidizing bacteria. Adv. Microbial Ecol. 8: 1–60.

9 Mancuso, C.A., P.D. Nichols and D.C. White. 1986. A method for the separation and characterization of archaebacterial signature ether lipids. J. Lipid Res. 27: 49–56.

10 Martz, R.F., D.I. Sebacher and D.C. White. 1983. Biomass measurement of methane forming bacteria in environmental samples. J. Microbiol. Methods 1: 53–61.

11 Milde, K., W. Sand, W. Wolff and E. Bock. 1983. Thiobacilli of corroded concrete walls of the Hamburg sewer system. J. Gen. Microbiol. 129: 1327–1333.

12 Nichols, P.D., J.B. Guckert and D.C. White. 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. Methods 5: 49–55.

13 Nichols, P.D., J.M. Henson, C.P. Antworth, J. Parsons, J.T. Wilson and D.C. White. 1986. Detection of a microbial consortium including type II methanotrophs by use of phospholipid fatty acids in an aerobic halogenated hydrocarbon-degrading soil column enriched with natural gas. Environ. Toxicol. Chem. 5: in press.

14 Nichols, P.D., W.R. Mayberry, C.P. Antworth and D.C. White. 1985. Determination of monounsaturated double bond position and geometry in the cellular fatty acids of the pathogenic bacterium *Francisella tularensis*. J. Clin. Microbiol. 21: 738–740.

15 Nichols, P.D., G.A. Smith, C.P. Antworth, R.S. Hanson and D.C. White. 1985. Phospholipid and lipopolysaccharide normal and hydroxy fatty acids as potential signatures for the methane-oxidizing bacteria. FEMS Microbiol. Ecol. 31: 327–335.

16 Parker, C.D. 1945. The corrosion of concrete. I. The isolation of a species of bacterium associated with the corrosion of concrete exposed to atmospheres containing hydrogen sulphide. Aust. J. Exp. Biol. Med. Sci. 23: 81–90.

17 Parker, J.H., G.A. Smith, H.L. Fredrickson, J.R. Vestal and D.C. White. 1982. Sensitive assay, based on hydroxy-fatty acids from lipopolysaccharide lipid A for gram negative bacteria in sediments. Appl. Environ. Microbiol. 44: 1170–1177.

18 Sand, W. and E. Bock. 1984. Concrete corrosion in the Hamburg sewer system. Environ. Technol. Lett. 5: 517–528.

19 Sand, W., E. Bock and D.C. White. 1984. Role of sulfur-oxidizing bacteria in the degradation of concrete. Corrosion 84, pp. 1–13, National Association of Corrosion Engineers, Houston, TX.

20 Smith, G.A., J.S. Nickels, B.D. Kerger, J.D. Davis, S.P. Collins, J.T. Wilson, J.F. McNabb and D.C. White. 1986. Quantitative characterization of microbial biomass and community structure in subsurface material: A prokaryotic consortium responsive to organic contamination. Can. J. Microbiol. 32: 104–111.

21 White, D.C. 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. In: Microbes in Their Natural Environments (Society for General Microbiology Symposium 34) (Slater, J.H., R. Whittenbury and J.W.T. Wimpenny, eds.), pp. 37–66, Society for General Microbiology, New York.

22 White, D.C. 1984. Chemical characterization of films. In: Microbial Adhesion and Aggregation (Dahlem Konferenzen Life Sciences Research Report 31) (Marshall, K.C., ed.), pp. 159–176, Springer-Verlag, Berlin and New York.

23 White, D.C. 1986. Environmental effects testing with quantitative microbial analysis: Chemical signatures correlated with *in situ* biofilm analysis by FT/IR. Toxicity Assess. 1: 315–338.

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