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## Determination of microbial fatty acid profiles at femtomolar levels in human urine and the initial marine microfouling community by capillary gas chromatography-chemical ionization mass spectrometry with negative ion detection

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### Summary

Room temperature esterification with the electron capturing pentafluorobenzyl bromide in glass capillaries, with analysis by capillary gas-liquid chromatography coupled with chemical ionization mass spectrometry and negative ion detection in the selected ion mode, allowed detection and identification of fatty acids from microbial biofilms at the femtomolar level. This sensitivity was achieved without loss of specificity of the mass spectrometric analysis. The detection of all the fatty acids commonly associated with bacteria was quantitative and linearly related to their concentration at a sensitivity permitting detection of about 600 bacteria the size of *Escherichia coli*. With this technique it was possible to detect the characteristic 3-hydroxy fatty acid of the lipopolysaccharide lipid A of *E. coli* infecting human urine at concentrations representing 10<sup>4</sup> bacteria and define the community structure of the initial marine microfouling community attached to a teflon surface at concentrations below the detectability by gas chromatography with flame ionization detection.

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Key words: *Capillary gas chromatography* - *Escherichia coli* - *Fatty acid analysis* - *Femtomolar sensitivity* - *Initial fouling community* - *Negative ion mass spectrometry* - *Urinary tract infection*

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### Introduction

Analysis of the methyl esters of fatty acids derived from the phospholipids of microorganisms from biofilms, soils and sediments have provided a reproducible and quanti-

tative means to define the biomass and community structure of microbial assemblies [1]. Fatty acid methods have proved free of the distortions induced with requirements for quantitative removal of organisms from surfaces, or the selectivity introduced when organisms are required to grow on artificial media. Most of these methods take advantage of the extraordinary resolution obtainable with capillary gas-liquid chromatography (cGLC) utilizing fused silica columns. The usual detector in these studies is the flame ionization detector (FID) which has detection limits of about  $3 \times 10^{-12}$  mol [2]. Assuming 100  $\mu$ mol fatty acid/g dry wt in bacteria [3] and  $5.9 \times 10^{12}$  bacteria/g dry wt [4] the limits of detection with FID correspond to  $2 \times 10^5$  bacteria the size of *Escherichia coli*.

Although the methyl esters of fatty acids represent the most commonly utilized derivatives in cGLC, other derivatives may offer advantages. Recently, halogenated ester derivatives such as trichloroethyl [5] and pentafluorobenzyl (PFB) esters [6, 7] have been used to increase the sensitivity and selectivity of detection. These derivatives show a strong response to the electron capture detector (ECD) and should in principle allow an increase in sensitivity of  $10^2$  or  $10^3$ . In practice, the relatively low specificity of the ECD results in noise which decreases the actual sensitivity. Mass spectrometric detection can provide high specificity and, when operated in the selected ion mode (SIM), maintains sensitivity. A characteristic constituent of mycobacteria, tuberculostearic acid, has been detected in sputum samples in patients with pulmonary tuberculosis at the 20 pg level using electron impact (EI) ionization mass spectrometry (MS) with SIM [8]. The sensitivity in the analysis has been increased 10-fold by utilizing chemical ionization mass spectrometry (CIMS) [9].

The ionization mechanisms operating in resonance electron capture negative ion chemical ionization (NICI) MS are similar to those in ECD [10, 11]. NICIMS allowed a 10–100-fold increase in relative sensitivity over ECD without loss of the superior selectivity of the MS detector [11].

A facile derivatization procedure involving the room temperature formation of PFB esters has been described [12]. These derivatives show a single molecular ion related base peak with NICIMS and very little additional fragmentation [12].

In this study, using a modification of the room temperature PFB derivatization together with cGLC/NICIMS, fatty acids have been quantitatively defined in the ultra-micro range from the initial marine microfouling community and urinary tract infections.

## Materials and Methods

### Materials

Solvents were glass-distilled reagent grade and not redistilled before use. The 2,3,4,5,6-pentafluorobenzylbromide (purity >99%) was from Fluka AG, Buchs, Switzerland. The fatty acid standards: 3R-hydroxynonanoic acid (3-OH 9:0), 3S-hydroxydodecanoic acid (3-OH 12:0), 2R-hydroxytetradecanoic acid (2-OH, 14:0), 12S-methyltetradecanoic acid (*a*-15:0), *cis*-hexadec-9-enoic acid (16:1 $\omega$ 7c), 14S-methylpentadecanoic acid (*i*-16:0), 14S-methylhexadecanoic acid (*a*-17:0), and *cis*-eicosa-5,8,11,14-tetraenoic acid (arachidonic acid, 20:4 $\omega$ 6c) were from the Lund laboratory collection (purity

>98%). (Fatty acids in this study are designated as the number of total carbon atoms: number of unsaturations with the position of the double bond closest to the aliphatic ( $\omega$ ) end of the molecule indicated with the configuration 'c' for *cis* and 't' for *trans*. Prefixes 'a' for anteisobranching, 'i' for isobranching, 'Br' for other methyl-branching, ' $\Delta$ ' for cyclopropane rings, and OH for hydroxyl with the position indicated are utilized.) The bacterial fatty acid mixture was from Supelco Inc. (Bellefonte, PA, USA) order number 4-5436, lot number LA11091.

#### *Urine samples*

One ml of urine from a patient with significant bacteriuria ( $>10^5$  organisms/ml) caused by *E. coli* and one ml from a healthy person were centrifuged at 1000 rpm for 15 min to remove sediment. The supernatants were recentrifuged at 17000 rpm for 30 min and the pellets collected for analysis.

#### *Marine initial fouling community*

Chloroform-extracted, autoclaved teflon strips (100 cm $\times$ 4 cm, Modern-Plastics, Hialeah, FL, USA) were exposed to flowing in situ seawater (temperature, 17°C, salinity, 30 p.p.t) in a darkened tank at the Florida State University Marine Laboratory, Turkey Point, FL, USA (29° 54.0'N, 84° 37.8'W). The surfaces were exposed over 5 days (November 16–21, 1983) and replicates were removed each day. After 2 days of exposure, excess silt built up on the surfaces due to a local storm. This was removed by a single pass through the seawater tank prior to lipid extraction for the surfaces removed on days 3–5.

#### *Lipid extraction*

The teflon surfaces were immediately extracted in a single phase system (chloroform: methanol: phosphate buffer, 1:2:0.8, v/v/v) [13]. After 24 h, chloroform and distilled water were added to form two phases (final solvent volume ratios, chloroform: methanol: water/buffer, 1:1:0.9). The phases were allowed to separate for 24 h and the total extractable lipids were removed and filtered through Whatman 2V filter paper, dried in vacuo, transferred to test tubes with teflon-lined screw caps, dried under a stream of dry nitrogen, and stored at -20°C until further analysis.

#### *Fatty acid recovery*

The pellets from the urine and standard bacterial fatty acid methyl esters were hydrolyzed with mild alkali to prevent loss of cyclopropane or hydroxy fatty acids [14]. Typically, 2 mg of sample was allowed to react with 1 ml of 15% NaOH in a 1:1 (v/v) methanol: water mixture heated at 80°C for 30 min in a test tube with a teflon-lined screw cap. After cooling, 2 ml distilled water and 1 ml hexane were added and the two-phase system vigorously shaken. The organic phase was removed. Dilute HCl was added until the aqueous phase was pH<2 and the free fatty acids extracted with two 1 ml portions of methylene chloride. The combined organic phases were washed with distilled water and evaporated to dryness with a stream of nitrogen at <40°C.

The total lipid from the initial fouling community was fractionated by silicic acid column chromatography [15]. The phospholipid after elution with methanol was col-

lected, dried under a stream of dry nitrogen, and hydrolyzed in test tubes with teflon-lined screw caps with 1 ml of freshly made 5% potassium hydroxide in methanol:distilled water (80:20, v/v) at 60°C for 60 min. Non-saponifiable phospholipids were removed with three washes of hexane:chloroform (4:1, v/v). The aqueous phase was acidified (pH<2) with 6 M hydrochloric acid, then re-extracted three times with hexane:chloroform (4:1, v/v) to recover the free fatty acids. The organic fractions were pooled in test tubes, dried under a stream of dry nitrogen, and transferred in hexane to pre-extracted capillary tubes (40 mm×2 mm, i.d.). The solvent was removed in an evacuated desiccator. The desiccator was then flushed with argon prior to opening and the capillary tubes immediately sealed for shipment to Lund.

#### *Derivatization*

The free fatty acids from the urine pellets or standard bacterial fatty acid mixture were dissolved in 30  $\mu$ l of acetonitrile. Then 10  $\mu$ l of 35% pentafluorobenzylbromide (PFB bromide) in acetonitrile was added followed by 10  $\mu$ l of triethylamine. After 10 min at room temperature (23°C), the mixture was dried in an evacuated desiccator. The derivatives were dissolved in 5  $\mu$ l of hexane and 1  $\mu$ l injected into the cGLC/MS. In the initial fouling experiments, the sealed capillary tubes were opened and derivatization with PFB bromide performed using one-tenth the volumes used in the test tubes.

#### *Gas chromatography*

cGLC studies of the derivatization procedure were performed using a Varian 3700 GLC equipped with FID and an all-glass splitless injection system. The capillary column was a 25 m fused silica column (0.2 mm, i.d.) deactivated with octamethylcyclotetrasiloxane (D4) and statically coated with SE-54. Hydrogen at a flow rate of 4 ml/min served as carrier gas and nitrogen at 30 ml/min as make-up gas. The chromatograph was operated at an injector temperature of 250°C and a detector temperature of 275°C. Injections were made with the oven at 100°C and after a delay of 2 min, the temperature was increased linearly at 4°/min to 270°C. The splitter was opened 1 min after injection.

#### *Mass spectrometry*

The Ribermag R10-10c quadrupole cGLC/MS/data acquisition system was used. The GLC was a Carlo Erba model 4160 with a capillary column as described above. Helium at a flow rate of 1 ml/min served as carrier gas. The chromatographic conditions were as above except that the initial oven temperature was 180°C. The methane reagent gas at 0.07 torr (purity >99.95%) was ionized with electrons of 94 eV and the ion source temperature was 100°C. The manual integration facility in the MS system's standard software was used for peak integration.

## **Results**

#### *Chromatography of PFB esters*

Figure 1 shows the response of PFB esters of the bacterial fatty acid mixture. The profile is identical to that of the methyl esters run with identical chromatographic

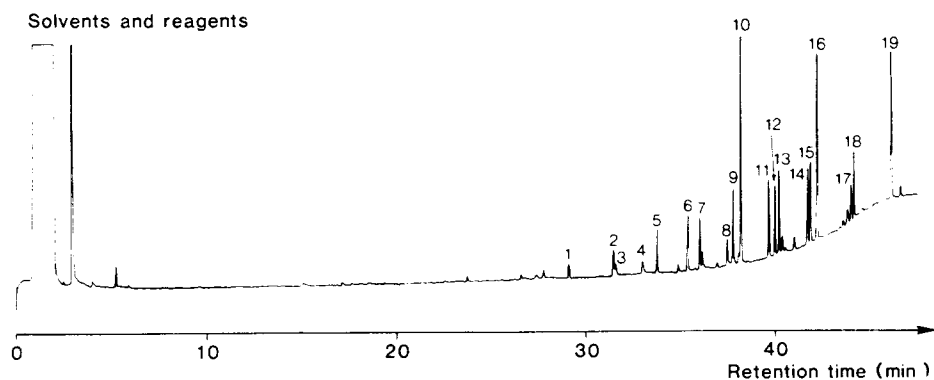


Fig. 1. Gas chromatogram of PFB esters of bacterial fatty acid mixture using FID with a splitless injection and hexane as the solvent. 1, 12:0; 2, 13:0; 3, 2-OH 12:0; 4, 3-OH 12:0; 5, 14:0; 6, *a*-15:0; 7, 15:0; 8, 3-OH 14:0; 9, 16:1; 10, 16:0; 11, *a*-17:0; 12, 17:0 $\Delta$ ; 13, 17:0; 14, 2-OH 16:0; 15, 18:1; 16, 18:0; 17, 19:0 $\Delta$ ; 18, 19:0; 19, 20:0.

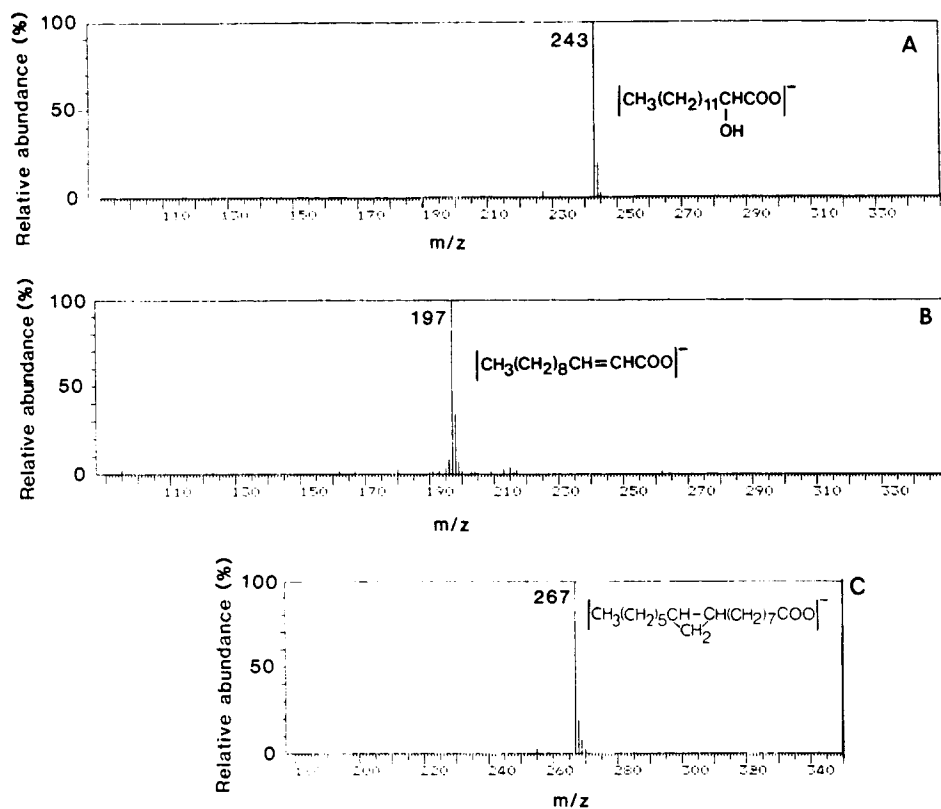


Fig. 2. NICl (methane) mass spectrum of PFB esters of: (a) 2-OH 14:0; (b) 3-OH 12:0; (c) 17:0 $\Delta$ .

TABLE 1  
SUMMARY OF NICI (METHANE) MASS SPECTROMETRIC CHARACTERISTICS OF PFB DERIVATIVES OF FATTY ACIDS

Short hand designation of acid	$M_r$ of derivative	Most abundant ion assignment of acid	$m/z$	%	Short hand designation of acid	$M_r$ of derivative	Most abundant ion assignment	$m/z$	%
3-OH 9:0	336	$[\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CHCOO}]$	155	100	16:0	436	$[\text{CH}_3(\text{CH}_2)_{14}\text{COO}]$	255	100
12:0	380	$[\text{CH}_3(\text{CH}_2)_{10}\text{COO}]$	199	100	2-OH 16:0	452	$[\text{CH}_3(\text{CH}_2)_{13}\text{CHOHCOO}]$	271	100
2-OH 12:0	396	$[\text{CH}_3(\text{CH}_2)_9\text{CHOHCOO}]$	215	100	17:0 $\Delta$	448	$[\text{CH}_3(\text{CH}_2)_8\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}]$	267	100
3-OH 12:0	396	$[\text{CH}_3(\text{CH}_2)_8\text{CH}=\text{CHCOO}]$	197	100			$\text{CH}_2$		
13:0	394	$[\text{CH}_3(\text{CH}_2)_{11}\text{COO}]$	213	100	$\alpha$ -17:0	450	$[\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)(\text{CH}_2)_{12}\text{COO}]$	269	100
14:0	408	$[\text{CH}_3(\text{CH}_2)_{12}\text{COO}]$	227	100	17:0	450	$[\text{CH}_3(\text{CH}_2)_{15}\text{COO}]$	269	100
2-OH 14:0	424	$[\text{CH}_3(\text{CH}_2)_{11}\text{CHOHCOO}]$	243	100	18:1	462	$[\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}]$	281	100
3-OH 14:0	424	$[\text{CH}_3(\text{CH}_2)_{10}\text{CH}=\text{CHCOO}]$	225	100	18:0	460	$[\text{CH}_3(\text{CH}_2)_{16}\text{COO}]$	283	100
<i>i</i> -15:0	422	$[\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)(\text{CH}_2)_{10}\text{COO}]$	241	100	19:0 $\Delta$	476	$[\text{CH}_3(\text{CH}_2)_8\text{CH}=\text{CH}(\text{CH}_2)_8\text{COO}]$	295	100
15:0	422	$[\text{CH}_3(\text{CH}_2)_{13}\text{COO}]$	241	100			$\text{CH}_2$		
16:1	434	$[\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}]$	253	100	19:0	478	$[\text{CH}_3(\text{CH}_2)_{17}\text{COO}]$	297	100
<i>i</i> -16:0	436	$[(\text{CH}_2)_2\text{CH}(\text{CH}_2)_{12}\text{COO}]$	255	100	20:4	484	$[\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_3\text{COO}]$	303	100
					20:0	492	$[\text{CH}_3(\text{CH}_2)_{18}\text{COO}]$	311	100

parameters except that the retention times are about 1.7-times longer than the corresponding methyl esters. The hydroxy fatty acids, which are particularly valuable as 'signatures' [16], showed only slight tailing associated with an underderivatized hydroxyl [17]. The hydroxyls were not derivatized to keep manipulations to a minimum and maximize sensitivity.

#### Mass spectrometry of PFB esters

The most important ions of the NICI mass spectra of the PFB esters of authentic standards and the bacterial fatty acid mixture are listed in Table 1. The mass spectra of three important PFB esters are illustrated in Fig. 2. The CI fragmentation produces the carboxylate ion from the loss of the PFB radical as the base peak with little additional fragmentation. The 2-OH PFB esters behave as unsubstituted or alkyl branched saturated and unsaturated esters. The 3-OH PFB esters lose a water molecule in addition to the PFB radical producing  $m/z \{M - (H_2CC_6F_5 + 18)\}^-$  (Fig. 2b). This was seen with the 3-OH 4:0, 3-OH 9:0, 3-OH 12:0 and 3-OH 14:0 and thus appears to reflect a general feature of these compounds.

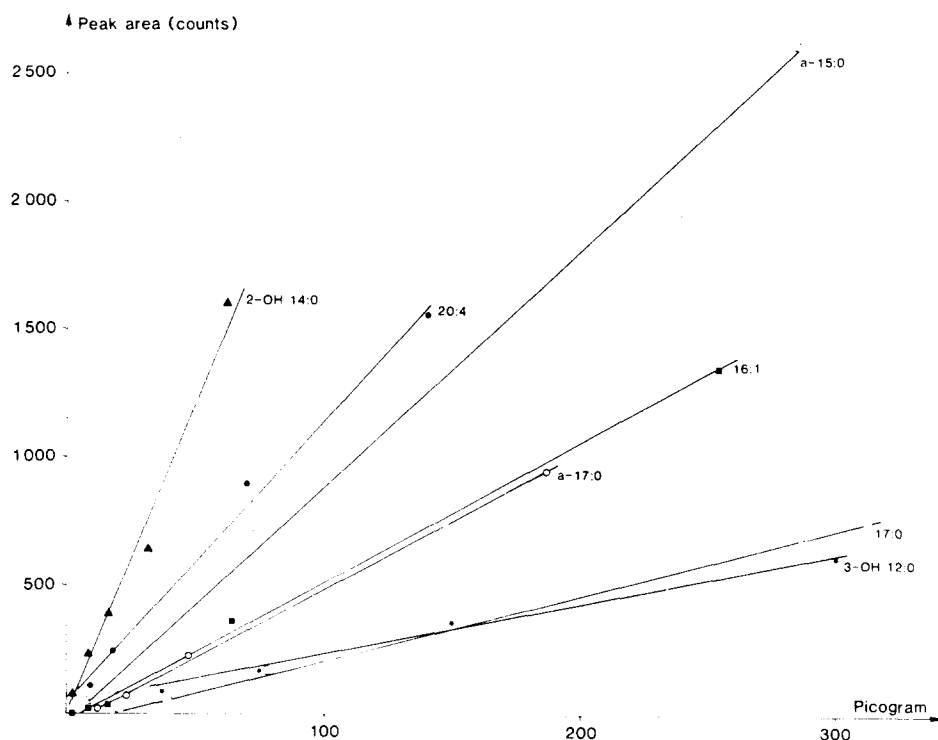


Fig. 3. Dose-response curves for a number of selected PFB esters of acids of microbiological relevance using NICI (methane) mass spectrometric SIM detection. Monitored ions: 2-OH 14:0,  $m/z = 243.20$ ; 20:4,  $m/z = 303.30$ ; a-15:0,  $m/z = 241.20$ ; 16:1,  $m/z = 253.20$ ; a-17:0,  $m/z = 269.30$ ; 17:0,  $m/z = 267.30$ ; 3-OH 12:0,  $m/z = 197.20$ .

*Quantification*

Dose response curves were constructed from the analysis of PFB esters of authentic or the commercial bacterial fatty acid sample (Fig. 3). The regression coefficient of the dose response curve for each of these PFB esters was  $r > 0.99$ . The dose response curves pass through the origin for all the acids except the cyclopropane 17:0 which may indicate a slight breakdown during the procedure.

The 3-OH 12:0, 2-OH 14:0 and 20:4 $\omega$ 6c PFB esters were measured with single ion monitoring, whereas the rest were assayed with 'simultaneous' multiple ion monitoring. This accounts for the steeper slopes for 2-OH 14:0 and 20:4 $\omega$ 6c. The 3-OH 12:0 apparently has a lower sensitivity than the other PFB esters.

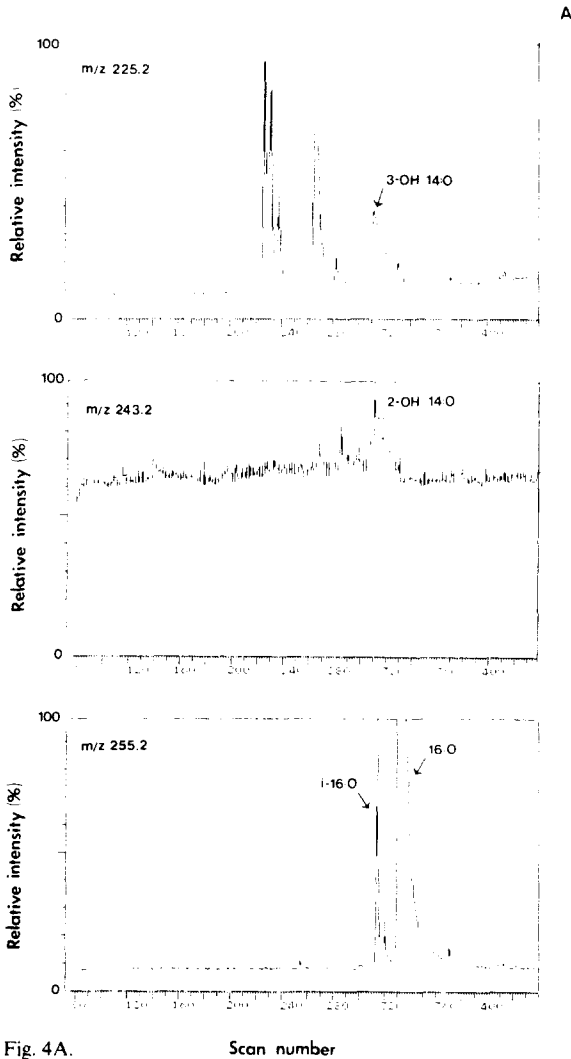


Fig. 4A.

Scan number



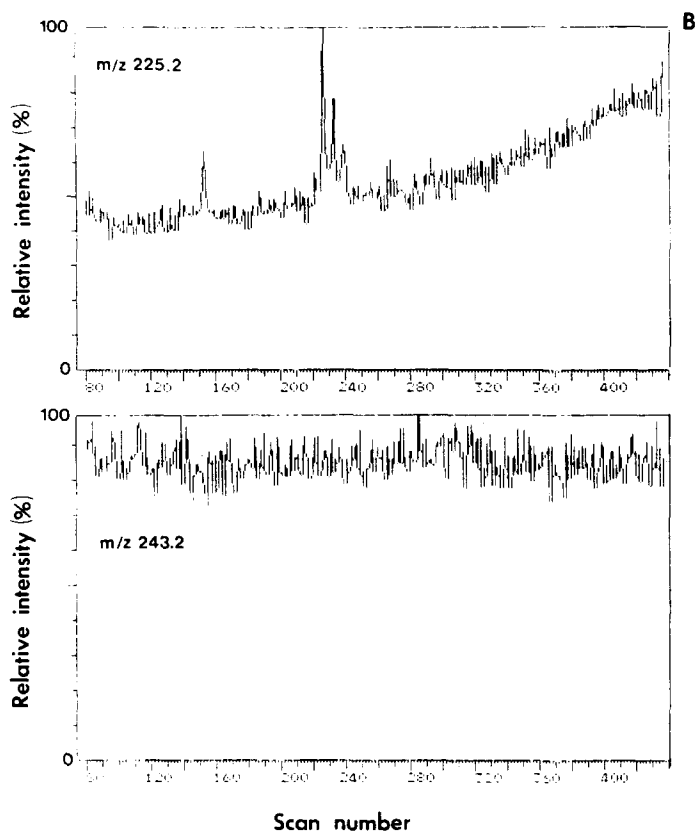


Fig. 4. Mass fragmentograms using NICI (methane) of PFB esters of acids in a hydrolysate of urine (A) from a patient with bacteriuria caused by *E. coli*, and (B) from a healthy person. SIM at  $m/z$  197.20,  $m/z$  225.20,  $m/z$  243.20 and  $m/z$  255.20 (*i*-16:0 as internal standard). The signals at  $m/z$  255.20 correspond to 1.1 pmol of *i*-16:0 acid.

#### Sensitivity

The detection limit for authentic 2-OH 14:0, and *a*-17:0 was approximately  $2 \times 10^{-15}$  mol using a signal-to-noise ratio of 2.

#### Urinary tract infection

Fatty acids of bacterial pellets from urine samples were prepared, the internal standard (*i*-16:0) added and then derivatized with PFB bromide. The chromatography was monitored at  $m/z$  197.20, 225.20, 243.20 and 255.20. Intense signals at  $m/z$  225.20 characteristic of 3-OH 14:0 (Fig. 4a, top panel), and at  $m/z$  243.20 at the retention time corresponding to 2-OH 14:0 with approximately 10% of the signal strength of the 3-OH 14:0 (Fig. 4A, middle panel), were present in the urine of the infected patient. No signals with the retention times for 3-OH 14:0 or 2-OH 14:0 were detected from the urine of healthy patients (Fig. 4B). The peak at  $m/z$  225.20 (Fig. 4B, top panel) eluted at scan number 270 which was much earlier than the 3-OH 14:0 (scan number 315, Fig. 4A, top panel).

Calculating from the signal of the internal standard of *i*-16:0 measured at  $m/z$  255.20 for  $1.1 \times 10^{-2}$  mol (Fig. 4A, bottom panel), the 3-OH 14:0 at  $m/z$  225.20 (Fig. 4A, top panel) represents about  $1.5 \times 10^{-12}$  mol.

*Initial marine microfouling community*

Replicates of the samples shown in Table 2 were analyzed as fatty acid methyl esters by GC with FID detection. No significant peaks were found above the limits of detection.

TABLE 2

ESTER-LINKED FATTY ACIDS OF THE PHOSPHOLIPIDS OF THE INITIAL MARINE MICROFOULING COMMUNITY OF TEFLON STRIPS EXPOSED TO RUNNING SEAWATER

Fatty acid	$m/z$	pmol per total surface area (800 cm <sup>2</sup> )				
		Day 1	Day 2	Day 3	Day 4	Day 5
<i>Br</i> -14:0 <sup>a</sup>	227	177	281	104	147	186
14:0	227	537	583	499	545	552
<i>Br</i> -15:0 <sup>a</sup>	241	89	63	43	52	54
<i>i</i> -15:0	241	374	419	230	349	430
<i>a</i> -15:0	241	187	209	115	174	215
15:0	241	342	369	292	342	367
3-OH 14:0	225	8	3	4	10	8
16:1	253	443	403	317	354	361
16:0	255	599	607	513	577	664
<i>i</i> -17:0	269	50	68	nd	52	81
<i>a</i> -17:0	269	55	74	63	48	101
17:0	267	110	90	73	39	68
Unknown	267	19	29	12	3	36
18:1	281	153	217	58	217	328
18:0	283	300	293	228	253	330
19:0	295	12	8	7	nd	11
20:0	311	11	15	6	7	21
Internal standard						
20:4 $\omega$ 6	303	5	4	7	7	4
Total bacterial fatty acids		3466	3731	2564	3169	3813
Total fatty acids (pmol/cm <sup>2</sup> )		4	5	3	4	5
Mol%						
Total saturates		52	50	60	54	51
Total unsaturates		17	17	15	18	18
Total branched		27	30	22	26	28
Total cyclopropane		4	3	3	1	2

<sup>a</sup> indicates the position of the branch is not known.

n.d., none detected.

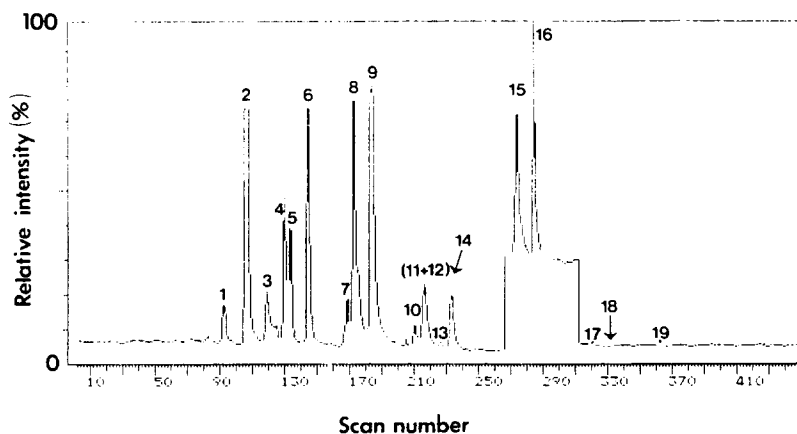


Fig. 5. Mass fragmentogram using NICI (methane) of PFB esters of acids in a hydrolysate of an initial marine microbial fouling community after exposure of the teflon surface for one day. SIM comprising five sets of ion parameters as listed in text. 1, *Br*-14:0; 2, 14:0; 3, *Br*-15:0; 4, *i*-15:0; 5, *a*-15:0; 6, 15:0; 7, 3-OH 14:0; 8, 16:1; 9, 16:0; 10, *i*-17:0; 11, *a*-17:0; 12, 17:0 $\Delta$ ; 13, unknown; 14, 17:0; 15, 18:1; 16, 18:0; 17, 19:0 $\Delta$ ; 18, 20:4 (internal standard); and 19, 20:0. The signals for 15:0 correspond to 1.4 pmol.

A typical SIM fragmentogram obtained from 1/250 of the sample of the biofilm formed on the first day of exposure using five combinations of different ion monitoring sets is reproduced in Fig. 5. Five consecutive sets comprising different ions of characteristic fatty acids from the lipids of microbial communities were monitored. The time events and ions were: 12 min 35 s:  $m/z$  227.20, 241.20, 243.20; 1 min 50 s:  $m/z$  225.20, 253.20, 255.20; 1 min 50 s:  $m/z$  267.30, 269.30; 2 min 5 s:  $m/z$  279.30, 281.30, 283.30; 10 min:  $m/z$  295.30, 303.30, 311.30 which correspond to the PFB esters of fatty acids as shown in Table 1. Comparison with corresponding fragmentograms of the reference bacterial fatty acid PFB ester mixtures and use of Table 1 made identification of most of the components straightforward. Incorporation of 20:4 $\omega$ 6 as an internal standard, monitored at 303.20, enabled approximate quantification of the fatty acids of the initial fouling communities (Table 2).

## Discussion

### *PFB esters*

The data of Fig. 1 show that the PFB esterification procedure produces no discrimination between even relatively unstable or sterically hindered acids with methylene or hydroxyl substituents. The room temperature procedure originally described by Strief and Murphy [12] was considerably simplified with the omission of the silicic acid chromatography step and could be adapted to capillary tubes for use with 4–10  $\mu$ l samples.

The mass spectrometry with NICI showed the carboxylate ions as base peaks with little fragmentation (Fig. 2). The 3-OH PFB esters apparently dehydrate, thus producing slightly less intense responses than 2-OH, branched, or unsaturated aliphatic esters. The

linear response that passes through the origin in the dose response curves (Fig. 3) indicates that there is little or no absorption or breakdown in the analytical system. There appears to be a slight loss of cyclopropane 17:0 PFB ester.

The sensitivity of the analysis of fatty acid PFB esters by NICIMS with SIM for authentic 2-OH 14:0 or *a*-17:0 was about  $2 \times 10^{-15}$  mol for a signal-to-noise ratio of 2. At this sensitivity an important 'signature' fatty acid that was recovered from ester-linkage in the phospholipid such as the *a*-15:0 could be utilized to detect about 600 bacteria like *Vibrio proteolytica*, an organism which was isolated from the marine initial microfouling community by John H. Paul of the University of South Florida. This calculation is based on the fact that *V. proteolytica* contains 20% of its total fatty acids as *a*-15:0 (unpublished data), bacteria contain  $10^{-4}$  mol of total fatty acid/g dry wt [3], and there are  $5.9 \times 10^{12}$  bacteria/g dry wt [4]. The analysis described herein provides a whole profile of fatty acids at this sensitivity that can be used to define specific components of the microbial community [1].

#### *Urinary tract infections*

The hydroxylated fatty acids covalently bound to the lipid A of the lipopolysaccharide (LPS) are sufficiently unusual to be excellent 'signatures' of specific groups of gram-negative bacteria [16].

The detection of 3-OH 14:0 and 2-OH 14:0 characteristic of enteric gram-negative bacteria in urine of a patient with urinary tract infection could define both that there was bacilluria and possibly provide an identification of the pathogen within several hours of receiving the sample. In the sample used for Fig. 4, the  $1.5 \times 10^{-12}$  mol of 3-OH 14:0 represents  $6.7 \times 10^5$  *E. coli*. *E. coli* contain  $14.4 \pm 2 \times 10^{-6}$  mol of 3-OH 14:0/g dry wt [16] with  $5.9 \times 10^{12}$  bacteria/g dry wt [4]. The 1 ml of urine was diluted 1/100 prior to derivatization so the sample represents an infection with  $6.7 \times 10^7$  bacteria/ml. With femtomolar sensitivity, it should be possible to detect about 120 bacteria with the same LPS content. The pattern of LPS lipid A fatty acids could also provide the identification of the species [16].

#### *Initial marine microfouling community*

The fouling of surfaces exposed to seawater generates enormous economic problems. The initial event in the fouling sequence involves an immediate coating of the surface with a biopolymer that attracts bacteria that first bind reversibly then irreversibly to the surface. This bacterial film then attracts an increasingly complex fouling succession that builds on the initial bacterial biofilm [18]. Control of the initial microfouling community could possibly regulate the whole succession. This biomass and community structure of the microfouling film can be defined by the analysis of 'signature' fatty acids [19]. Increasing the sensitivity of the measurements of the fatty acid profile of this community provides deeper insight into the earliest phases of the process.

The biofilm attached to teflon strips exposed to running seawater did not change its community structure significantly between the first and second and the fourth and fifth day of exposure (Table 2). The decrease in fatty acid recovery on the third day of exposure may have resulted from the need to clean the sediment off the teflon strip. The sediment accumulated after storm-induced turbulence introduced sediment into the

seawater supply. The teflon strip was cleaned by a single pass through the seawater tank which may have removed the less tightly-bound bacteria. On this day there was a decrease in the total bacteria and decreased proportions of branched and monounsaturated fatty acids with higher proportions of saturated fatty acids in the phospholipids of the biofilm. This illustrates the power of the fatty acid analysis to quantitatively define the community structure of microbial biofilms. In the other samples, the biofilm maintained a stable density equivalent to  $2.5 \times 10^5$  bacteria the size of *E. coli*/cm<sup>2</sup>. With the sensitivity of the cGLC/NICIMS analysis of PFB esters, the effects of changing the seawater composition, the surface properties, and the shear environment of adhesion on the very initial phases of the attachment could be studied at bacterial densities approaching 100 bacteria/cm<sup>2</sup>.

Examining the fatty acid profile of the initial microfouling community shows a number of its properties. The absence of long chain polyunsaturated fatty acids indicates the lack of microeukaryotes in the initial microfouling film [20]. Several of the prominent fatty acids in this community are characteristic of bacteria: the 15 and 17 branched, the cyclopropane and the monounsaturated fatty acids with the unsaturation at the  $\omega 7$  position in the chain from the anaerobic desaturase pathway are found primarily in bacteria [2].

## Conclusions

The combination of rapid, room temperature derivatization and the specificity and sensitivity of chemical ionization with negative ion detection for PFB ester derivatives provides a highly sensitive and specific means of detecting characteristic fatty acid profiles that are recovered from the lipids of biofilms. This will be of great importance in defining the critical initial phases of contamination of air or water supplies, of infection of plants and animals, and the initiation of microbially-mediated biodeterioration processes such as biofouling and corrosion.

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